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THE UNIVERSITY OF ALBERTA

A GENETIC STUDY OF SYMBIOTIC NITROGEN FIXATION IN THE TROPICAL LEGUME *DESMODIUM*

by

© BRIAN ROY PINCHBECK

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have reed, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "A genetic study of symbiotic nitrogen fixation in the tropical legume, Desmodium" submitted by Brian Roy Pinchbeck in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Agronomy.

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ABSTRACT

A genetic study was initiated in the tropical legume Desmodium to establish whether there existed genetic variation in the symbiotic relationship between Desmodium and Rhizobium. Particular emphasis was placed upon effective vs. ineffective nodulation and quantitative variation for the estimates of nitrogen fixed.

Analysis of F_2 and backcross populations from two interspecific crosses suggested that a strain-specific ineffective nodulation interaction was conditioned by a dominant allele at a single locus.

Significant genetic variation was found among genotypes for all nitrogen fixation estimates. The major portion of the variation among genotypes was non-additive variation and was attributable to a significant Varieties vs. Crosses genetic component. The mean of the F_1 crosses was found to be 80% larger than the mean of the inbred parents indicating that most F_1 crosses exceeded their larger parent for the quantitative estimation of nitrogen fixation. Since inbreeding depression for forage yield is generally considered to be absent in the presence of adequate nitrogen, the F_1 hybrid vigor for the nitrogen fixation estimates was due solely to these differences when the plants were grown in a nitrogen limiting environment. The significance of these results was discussed.

Large cotyledons and juvenile leaves appeared to be controlled by dominant genes at different loci. Plants with large cotyledons or juvenile leaves fixed significantly more nitrogen than plants with small ones. Adjusting the quantitative estimates for differences due to cotyledon and juvenile leaf size produced a totally additive model for the inheritance of the estimates of nitrogen fixation. The non-additive variance for the unadjusted estimates could be divided into two components: (a) additive variation due to plant vigor, and (b) additive variation due to 'efficiency' of nitrogen fixation. A breeding program utilizing these additive components was discussed.

Genotype x environment interactions were of significant importance suggesting that study concerning quantitative variation for amount of nitrogen fixed should be conducted in different environments with various *Rhizobium* strains.

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INTRODUCTION

The development of tropical agriculture has recently commanded much interest in an effort to solve pending problems of world food shortages. The improvement of tropical legumes can contribute to this development through the improved symbiotic fixation of atmospheric nitrogen. It has been conceded that the amounts of nitrogen fixed by tropical legumes were generally less than for temperate legumes (Vincent, 1974). Since temperate and tropical legume species differed in the amount of nitrogen fixed, it would be pertinent to inquire as to whether genotypes within a species also differed. If genotypes differed significantly, can this variation within the host for symbiotic nitrogen fixation be exploited in a breeding program for the improvement of tropical legume species? It would be of particular interest to initiate a genetical study of the inheritance of symbiotic nitrogen fixation in the tropical legume Desmodium because of its great variation in the morphological differentiation of the numerous species (Bryan, 1966) and its potential as a forage legume (Bryan, 1969).

Norris (1956) postulated that the legume-Rhizobium symbiosis originated in tropical rain forests. As the legume adapted to the subtropical and temperate regions, the symbiotic interaction appeared to become more specific. Since the Rhizobium which nodulates Desmodium is a member of the "Cow-pea" group, a promiscuous interaction group of

Rhizobium, the Desmodium - Rhizobium symbiotic interaction may represent a more primitive form of symbiosis. However, Bryan (1966) reported that this interaction appeared to be specific as measured by numerous ineffective nodulations.

A. Generalized description of Desmodium.

Desmodium is a self-fertile tropical legume with a somatic chromosome number of 2n=22 (Rotar and Urata, 1967). Rotar and Urata (1967) reported collections of many different species from tropical countries including two species from Canada. McWhirter (1969) suggested that the center of origin of Desmodium was in South America. The taxonomic identity of this genus is uncertain (McWhirter, 1969) and voucher specimens have been deposited by some Desmodium researchers (Rotar and Urata, 1967; McWhirter, pers. com.) at the Harvard University Herbarium for identifications by Dr. Bernice Shubert, Arnold Arboretum, Harvard University.

Four Desmodium species have attracted attention as forage legumes. These species are D. sandwicense E. Mey, D. canum (Gmel), D. aparines (Link) D.C.* and D. uncinatum (Jacq) D.C.. D. aparines and D. uncinatum have been released as the commercial cultivars Greenleaf and Silverleaf desmodium, respectively, in Australia (Imrie, 1973) as they are much more productive and robust (stem length is 5 to 20 meters) than either *D. canum* or *D. sandwicense*. *D. canum* has attracted attention in Hawaii in pasture swards (Younge, *et al.*, 1964). *D. sandwicense* is the least robust of these four species with the stem length rarely exceeding two meters. McWhirter (1969) reported that F_1 heterosis for forage yield was not present in inter or intra-specific *Desmodium* crosses while Rotar and Chow (1971) indicated that the internode length of a two-species F_1 cross was intermediate to its two parents. These data suggest that *Desmodium* is not affected by inbreeding depression for forage yield.

---

*Also called D. intortum (Mill) Urb.

Than Aung (1970) described *D. sandwicense* as a postrate herb whereas Rotar and Chow (1971) observed an erect growth habit. The stem color has been reported by Rotar *et al.*, (1967) to be either red-brown or green. The segregation of this character is 3 red-brown to 1 green (Hutton and Gray, 1967). Than Aung (1970) found the stems to be green with red pigmentation at the nodes. The stem was found to be round (Rotar, *et al.*, 1967) or trisulcate (Than Aung, 1970) in cross section. The stems are pubescent with slightly hispid hairs (Than Aung, 1970) or glandular (Rotar *et al.*, 1967). Rotar *et al.* (1967) found the internode length varied from 2.9 to 3.2 cm which was shorter than *D. uncinatum* or *D. aparines*. These authors believe that the internode length is controlled by multiple genes since the interspecific cross with *D. aparines* was intermediate to the parental phenotypes.

The leaves of *Desmodium* are alternate, entire and trifoliate with the terminal leaflet being larger. McWhirter (1969) reported a dominant polyphyllous leaf gene. The leaf form of *D. sandwicense* is lanceolate with acute apices (Rotar *et al.*, 1967) or ovate and cordate at the base (Than Aung, 1970). Rotar *et al.* (1967) found the length/width ratio of the sixth leaflet from the flower bud to range from 1.79 to 1.89 and the leaf area (length x width) to be from 8 to 12 sq cm. Than Aung (1970) found the leaf area to vary from 8 to 37 sq cm for all terminal leaflets. Hutton and Gray (1967) reported that the leaflets of *D. sandwicense* and *D. uncinatum* contain a silver-grey marking along the midrib. This character is dominant to the plain leaf and segregated as a single locus. The spotted leaf character common in *D. aparines* is occasionally found in *D. sandwicense* (McWhirter, pers. com.). Hutton and Gray (1967) reported that this character was dominant to the plain leaf and segregated with a 3:1 ratio in the F_2 generation. McWhirter (pers. com.), however, suggested that the spotted leaf character is a complementary dominant gene system. The spotted leaf was found to be closely linked with the stem color (Rotar *et al.*, 1967).

The flowering habit of *D. sandwicense* is relatively indeterminant and differs from the *D. uncinatum* and *D. aparines* which flower in response to short days (Rotar, *et al.*, 1967). The above authors also report that *D. sandwicense* will outcross at a rate of 18%. McWhirter (pers. com.) suggested that *Desmodium* is highly self pollinated in the wild and estimates the outcrossing to be about 20%. He indicated that most collections give an impression of being rather highly inbred.

The flowers of Desmodium are borne on terminal or axillary racemes. The basal flowers of the raceme are first to open and flowering usually continues for eight days (Rotar et al., 1967) with about five flowers opening each day. Preceding the opening of the flowers, the raceme elongates rapidly separating the flowers. The petals elongate beyond the sepals the day before the flower opens. The anthers dehise at this time and the pollen is mature. The female parts of the flower mature the following morning when the standard becomes erect. The pistil is held between the keel florets and when tripped it halts midway to the standard without striking it. The pollen is discharged in a cloud about the stigma. Hutton (1960) reported that D. sandwicense trips spontaneously while Rotar et al. (1967) state that it will pollinate without tripping. McWhirter (pers. com.) reported that the pistil is 1 to 2 mm longer than the stamens and that pollination will not occur until tripped or disturbed. Following pollination, the flower wilts rapidly with the standard folding over the stigma.

The flower color in Desmodium is polymorphic varying from white to purple (Than Aung, 1970). The purple flower color is dominant to white and this character shows a complementary dominant gene inheritance in crosses of certain white flowered species (D. sandwicense and D.

aparines) (McWhirter, pers. com.).

The seed pods of *Desmodium* are distinctly articulated and contain an average of 6.3 seeds per pod (Rotar *et al.*, 1967). The pods are covered with short hairs and stick readily to clothing or fur. The seeds are kidney-shaped and light brown in color. The 1000-kernel weight of *D. sandwicense* was 3.5 gm falling between *D. uncinatum* (4.0 gm) and *D. aparines* (1.8 gm) (Rotar and Urata, 1966).

The germination for machine harvested seed is 99% but germination is reduced when hand harvested. Hand harvested seeds may be scarified by chipping the testa (McWhirter, pers. com.) or immersion in concentrated sulfuric acid for 15 min (B. Groff, pers. com.). Following scarification, germination is normal.

McWhirter (1963) first reported successful crosses between *D. sandwicense* and *D. aparines*. Hutton and Gray (1967) reported successful crosses between *D. sandwicense* and *D. uncinatum* and *D. uncinatum* and *D. aparines*. They found no gross cytological barriers; however, incompatabilities were found by McWhirter (pers. com.) when specific lines were used. McWhirter (pers. com.) suggested that *D. aparines* be used as a bridging species.

Rotar and Chow (1971) found that pod formation was highest when *D. sandwicense* was the female parent but much reduced when it was the male parent. McWhirter (pers. com.) also found a reduced pod set when *D. sandwicense* was the male parent. Rotar and Chow (1971) found crosses between *D.*

uncinatum and *D. aparines* to be low for pod set and they concluded that physiological factors may be influential. Pollen abortion was highest in the *D. sandwicense* x *D. aparines* crosses and was lowest in the *D. sandwicense* x *D. uncinatum* crosses (Rotar and Chow, 1971). Rotar and Chow (1971) concluded that *D. sandwicense* was more closely related to *D. uncinatum* than to *D. aparines*. They reported that pod set was negatively correlated with percentage pollen abortion (r = .74, df = 21). McWhirter (pers. com.) reported that morphological differentiation was correlated with genetic divergence. He concluded that *D. uncinatum* was morphologically distinct from *D. sandwicense* and *D. aparines*.

Chow and Crowder (1972) report a cross between *D. canum* and *D. uncinatum*. They conclude that *D. canum* is genetically separate from *D. sandwicense*, *D. uncinatum* and *D. aparines* .

B. Genetic studies of symbiotic nitrogen fixation.

The study of symbiotic nitrogen fixation has been of considerable interest to agriculture for numerous years and this area has recently been comprehensively reviewed (Quispel, 1974; Hardy and Gibson, 1977). The limitation of genetical studies of the inheritance of symbiotic nitrogen fixation was of major concern (Dart, 1974). The majority of the genetical studies involved the study of major genes conditioning non-nodulating or ineffective nodulation interactions. Few genetical studies have been reported giving results for plant variation for total nitrogen fixed.

Major genes conditioning strain-specific nodulation have been reported in alfalfa (*Medicago sativa* L.) (Burton and Wilson, 1939; Gibson, 1962), red clover (*Trifolium pratense* L.) (Nutman, 1954), subterranian clover (*T. subterraneum* L.) (Gibson, 1964), soybean (*Glycine max* L. Merril) (Williams and Lynch, 1954; Caldwell, 1966; Vest, 1970; Vest and Caldwell, 1972), and peas (*Pisum sativum*) (Holl, 1975; Lie *et al.* , 1976) . The major genes appeared to be of two types: (a) recessive genes with modifiers, or (b) dominant genes. The investigations in *Trifolium* suggested that strain-specific ineffective nodulation was controlled by a single recessive gene modified by recessive suppressor genes which restored effectiveness (Nutman, 1954; Gibson, 1964). Single dominant genes (Rj_2, Rj_3 and Rj_4) were reported in soybeans conditioning strain-specific ineffective nodulation (Caldwell, 1966; Vest, 1970; Vest and Caldwell, 1972) while the recessive gene (rj_1)* conditioned a universal non-nodulation interaction in soybeans (Williams and Lynch, 1954).

Vest *et al.* (1973) classified the soybean plants homozygous for the Rj alleles (cultivars - Hardee, Hill) as nodulated-ineffective since the plants exhibited small, white cortical proliferations at the node sites. They concluded that infection had taken place. However, nitrogen was not being fixed. Vest et al. (1973) reported that Rj_2 and Rj_3 appeared to be linked yet both were independent of rj_1. Although Rj_2 and Rj_3 were similar phenotypically, producing numerous small, white cortical proliferations, they appeared to differ in the serological properties of the ineffective strains. Rj_2 was ineffective with most strains of the cl and 122 serogroups while Rj_3 was ineffective with only one particular strain. Caldwell et al. (1966) performed reciprocal grafts of the cultivars Hardee (Rj_2, Rj_2) and Lee (rj_2, rj_2) and reported that the root genotype dictated the nodulation interaction.

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*Designated *no* by Williams and Lynch (1954).

The ineffective response for the Rj_4 gene (Hill x strain 61) appeared to be a specific response in the sense of the Rj_3 gene. The Rj_4 gene produced only a few small, white cortical proliferations since nodule abortion probably occurred at a very early stage of development (Vest and Caldwell, 1972).

A universal non-nodulation gene has also been reported for peas. Holl (1975) and Holl and LaRue (1975) studying crosses of an Afghanistan cultivar (non-nodulating) reported two genes affecting nodulation and fixation. Holl (1975) reported that the Sym_2 locus (nodulation) and the Sym_3 locus (fixation) interacted to give four phenotypes for fixation. Plants with the genotype Sym_2 --,Sym_3 -- were classed as nodulating and fixing while plants with the genotype sym_2 sym_2, -- -- were non-nodulating and non-fixing. However, when the Sym_2 gene was combined with the homozygous recessive condition for the sym_3 gene the resulting combination conditioned differing interactions. If the Sym_2 gene was heterozygous the resulting interaction was nodulation but no fixation, whereas, a homozygous dominant condition for the Sym_2 gene resulted in nodulation and low fixation. The Sym_2 locus conditioned nodulation and segregated 3 nodulating to 1 non-nodulating phenotypes.

Lie et al. (1976) reported a strain-specific, temperature-sensitive non-nodulation interaction in the pea cultivar, Iran. The authors indicated that nodulation occurred only when the root was subjected to favorable nodulation temperatures (26 C) for the second and third day following inoculation. Adverse temperatures during this period reduced nodulation whereas adverse temperatures before or after the critical period had no effect. Lie et al. (1976) reported that this strain-specific, temperature-sensitive ineffective nodulation interaction was conditioned by a dominant allele at a single locus.

The study of symbiotic nitrogen fixation has generally dealt with qualitative interactions relating to nodulation vs. non-nodulation. However, genetical studies have been reported for quantitative variation for estimates of nitrogen fixation. Burton and Wilson (1939) reported that varieties of alfalfa differed significantly for total nitrogen fixed when inoculated by several strains of R. meliloti. Conversely the authors also noted significant differences when strains were compared as well as specific host-strain interactions. These data suggested quantitative differences among hosts and among strains. Burton and Wilson (1939) also noted that the results tended to be erratic and attributed these deviations to environmental factors. Later work in alfalfa by Gibson (1962) confirmed genetic variation of the host-strain interaction. He noted that the Canadian cultivar Rambler was inefficient when inoculated with Australian strains of R. meliloti yet was efficient when inoculated with Canadian strains. Gibson (1962) suggested that the M. falcata parentage of Rambler may be, in part, responsible for this inefficiency. Recently Seetin and Barnes (1977) reported genetic variation in alfalfa for quantitative nitrogen fixation estimated by the acetylene reduction assay and dry weight accumulation. These authors indicated that the alfalfa clones expressed F_1 heterosis and inbreeding effects for the estimates of nitrogen fixation. Duhigg et al. (1978) reported that F_1 progeny from Mesilla alfalfa plants selected for high acetylene reduction values expressed significantly greater acetylene reduction values than the parental population. Significant results were not reported for the low selection line.

In recent research with field beans (Vicia faba), El-Sherbeeny et al. (1977a,b) reported genetic variation among Rhizobium leguminosarum strains and among field bean lines, respectively. Mytton et al. (1977) indicated that the genetic variation for lines or strains was found to be minor in comparison to the line by strain interactions. Mytton et *al*. (1977) partitioned the genetic variance into three components; namely, (a) the additive genetic effect of the host genotype, (b) the additive genetic effect of the strain genotype, and (c) the non-additive effect of the host by strain interactions. They indicated that 73% of the phenotypic variation could be directly attributed to host strain interactions and concluded that simultaneous selection for a specific host-strain interaction would be most effective.

Mytton (1975), studying white clover (*Trifolium repens*) symbiosis, found that host variation for nitrogen fixation was larger than for differences among strains. He concluded that only effective strains had been collected thereby reducing genetic variation. However, Mytton (1975) did report significant host x strain interactions. Strains which were selected from one plant generally entered into a more productive interaction with relatives of that plant than with non-related plants suggesting selective hosts.

Minchin *et* *al*. (1978) also found genetic variation among cultivars of cowpea (*Vigna unguiculata*)-*U*) *but not among* Rhizobium strains. The above authors, unlike Mytton *et al*. (1977), concluded that any breeding program should concentrate on the macrosymbiont.

Quantitative assessments of the amounts of nitrogen fixed had been measured in earlier experiments by total dry weight accumulation of the shoots when plants were grown in nitrogen free containers (Gibson, 1962; Vincent, 1970). The

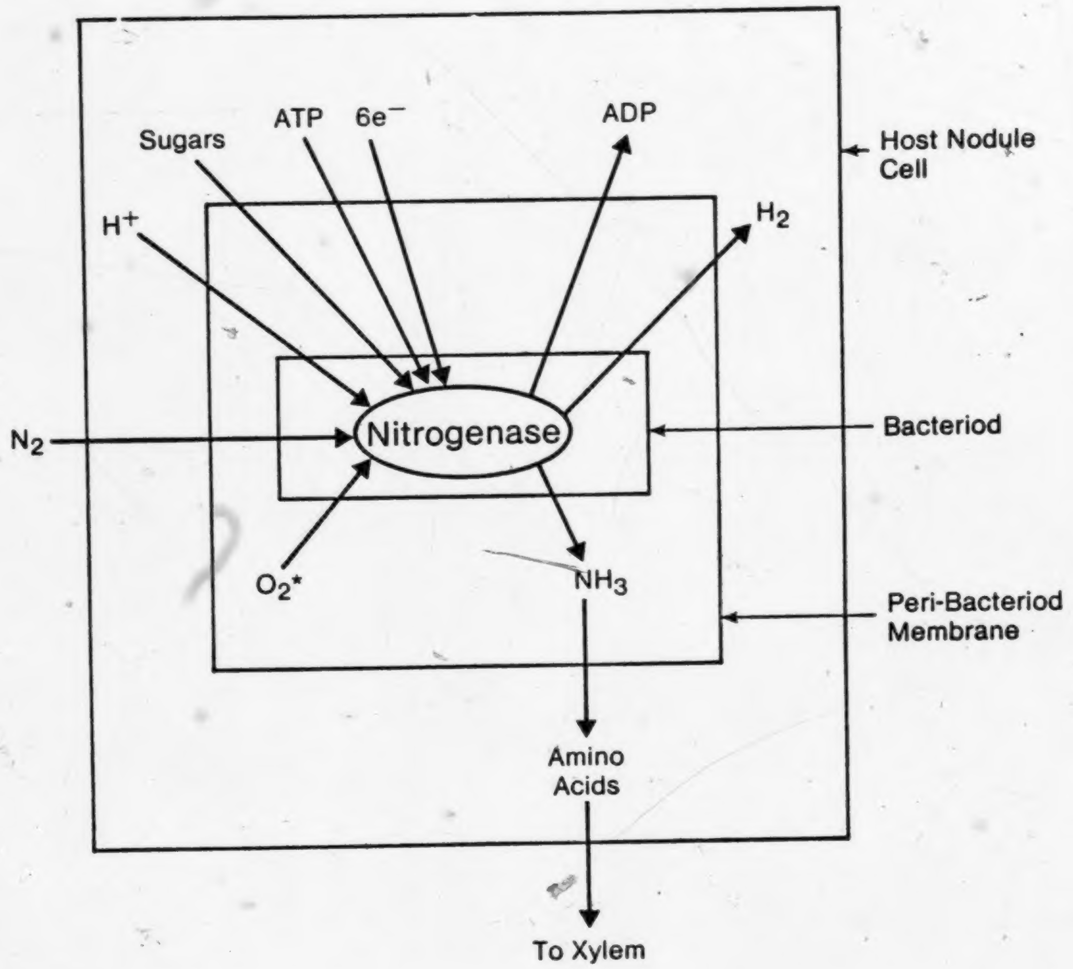
^{15}N method (Burris and Wilson, 1957) and, recently, the acetylene reduction assay (Hardy *et al.*, 1968) have been developed as assays of the activity of the enzyme nitrogenase (Figure 1). Of these two assays, the acetylene reduction assay has been most popular because it was simple, rapid and more sensitive than the ^{15}N method; but it was an indirect approximation (Burris, 1974). Since its introduction, the acetylene reduction assay has appeared in many publications measuring nitrogenase activity in intact nodules, nodule prepared cell-free extracts, field experiments, as well as marine and fresh-water habitats (Quispel, 1974).

The reduction of acetylene to ethylene by nitrogenase was independently discovered by Schollhorn and Burris (1966) (reviewed by Burris, 1974) and Dilworth (1966). Its usefulness as an index of nitrogenase activity was immediately realized by Hardy *et al.* (1968) who developed a method of measuring nitrogenase activity for field experiments. The relationship between acetylene and N_2 reduction may be represented as:

$HC --- CH + 2e^- + 2H^+ = H_2C --- CH_2$

$N --- N + 6e^- + 6H^+ = 2NH_3$

These formulae suggested that the conversion rate for N_2 to NH_3 was one-third that of C_2H_2 to C_2H_4 since acetylene reduction required two electrons while N_2 reduction required six. Burris (1974) reported that the conversion factor of 1/3 was not generally found but was much closer to 1/4 and


Sugars
ATP
$6e^-$
ADP
Host Nodule Cell
H^+
H_2
N_2
Nitrogenase
Bacteriod
O_2*
NH_3
Peri-Bacteriod Membrane
Amino Acids
To Xylem

*Leghemoglobin Transported

Figure 1. A diagramatic representation of the symbiotic nitrogen fixation pathway in the host nodule cell.

Burris suggested a comparison of the acetylene reduction essay with the ^{15}N method to determine an exact conversion factor when required for a particular experiment.

Recent studies with the acetylene reduction assay in soybeans suggested that nodule activity (C_2H_4) was determined by the physiological stage of the plant (Lawn et al., 1974). Lawn and Brun (1974) found that the nodule fresh weight and acetylene reduction activity for soybeans reached a maximum near the termination of flowering and declined rapidly during the onset of the pod-filling stage. The authors attributed this decline to a competition by the pods for available assimilate. Additional studies with reciprocal grafts in soybeans indicated there were significant differences among shoot and root genotypes as well as for sampling dates as measured by the acetylene reduction assay (Lawn and Brun, 1974). Significant shoot genotype x sampling date interactions were found for all characters studied while root genotype x sampling date interactions were not significant with the exception of the photosynthetic rate. There were significant shoot genotype x root genotype interactions for nodule number and nodule size. Nitrogenase activity (C_2H_4) for the shoot genotype means was significantly correlated with nodule fresh weight, root dry weight, shoot:root ratio, photosynthesis and shoot dry weight. However nitrogenase activity (C_2H_4) for root genotype means was not significantly correlated with any of the above characters. Lawn et al. (1974) concluded that root genotypes may differ in their ability to efficiently utilize the photosynthate assimilated during symbiotic nitrogen fixation. They also suggested that the root genotype controlled nodule initiation and nodule size which were found to be significantly negatively correlated with the root genotype effect on nodule number.

Dart (1974) reported responses to selection in several *Trifolium* species for number of nodules per plant, time taken to form the first nodule and effectiveness of the nodules. These characteristics were also affected by the *Rhizobium* strain used. Dart (1974) concluded that plant breeding for increased nodulation was of significant potential.

C. Objectives.

It was the objective of this study of the inheritance of symbiotic nitrogen fixation in *Desmodium* to establish whether or not there exists genetic variation in the processes of the symbiotic relationship between host plant and *Rhizobium* with particular emphasis upon effective vs ineffective nodulation and the quantitative variation in total symbiotic nitrogen fixed per plant. It was also of interest to determine the genetic basis for such differences, if disclosed. The dry weight accumulation in a bottle jar (Leonard jar assembly) and the acetylene reduction assay were assessed to determine their potential as plant breeding tools.

MATERIALS AND METHODS

A. Outline.

In this study of the inheritance of symbiotic nitrogen fixation in Desmodium, experimentation was conducted at three levels. These levels, in increasing order of interest, have been broadly defined as methodology, qualitative genetic variation, and quantitative genetic variation (Table 1). The methodological studies encompassed the background studies necessary for the development and improvement of the technique of studying symbiotic nitrogen fixation. Experiments in qualitative genetic variation were conducted to determine the genetic basis for ineffective/effective

Table 1. Levels of study of the inheritance of symbiotic nitrogen fixation in the host.

		Comments:
Level 1	Methodology	Preliminary studies How? What? Where? Improvements? When?
Level 2	Qualitative Genetic Variation	
Host-strain interactions* effective	ineffective	Which? (Major genes?)
Level 3	Quantitive genetic variation	
Fixation "efficiency"* efficient	inefficient	How much? (Minor genes?)
Ultimate goal		Why? Selection Super-fixing inbred lines or hybrids

*May also be modified by strain manipulation.

nodulation interactions. The experiments involved the testing of the observed segregation ratios in F_2 and backcross populations for a goodness of fit to an expected segregation ratio.

The experiments involving quantitative genetic variation constituted the major interest in this study and relate to the investigation of the inheritance of total symbiotic nitrogen fixed per plant. The experimental designs which were chosen to establish and examine the genetic variation were: (1) a five-parent diallel cross, and (2) a cross between two inbred lines. The diallel cross consisted of inbred lines of D. sandwicense which were not selected for the characters under study. The cross between two inbred lines constituted crosses of D. sandwicense with two other species of Desmodium which were conspicuously different from D. sandwicense as well as a within D. sandwicense cross.

B. Materials.

1. Rhizobium strains.

To study one component of the host-strain interaction of symbiotic nitrogen fixation, it was necessary to hold the other component constant. Since the variation within the population created from crosses among Desmodium lines was of major interest, the Rhizobium component within each experiment has been held constant and was considered to be an environmental component. The two pure strains of

Rhizobium selected were strain A (CB627)* and strain B (CB1789)* which were isolated from D. aparines and D. sandwicense, respectively. These strains differed in growth properties. Both strains are classed as slow growing; however, strain A is considered to be relatively faster growing than strain B. Both strains are members of the "Cowpea" group of Rhizobium (Vincent, pers. com.) and strain A has been released in Australia as a commercial inoculum for Desmodium.

2. Desmodium lines.

The Desmodium lines used in this study are described in Table 2. D. sandwicense or its crosses were used in most experiments because it; (a) is diploid, self-fertile and readily crossed, (b) is relatively insensitive to photoperiodism and is easily maintained under glasshouse conditions, (c) has a robust seedling (desirable for culture in a bottle jar assembly), and (d) can be hybridized easily with other conspicuously variable and commercially important species of Desmodium (Than Aung, 1970). Prolonged inbreeding does not adversely effect plant vigor and inbreeding depression is generally considered to be absent (McWhirter, 1969; Rotar and Chow, 1971). These characteristics made D. sandwicense an excellent model organism for nitrogen fixation studies.

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*Commonwealth of Australia identification number.

Table 2. Description of the *Desmodium* lines involved in this study.

Study Identification	Species	Accession or Line Ident.	Source
Ds1	*D. sandwicense* E. Mey	CPI 18156	Dr. K.S. McWhirter, U. of Sydney
Ds2	*D. sandwicense* E. Mey	CPI 18163	Dr. K.S. McWhirter, U. of Sydney
Ds3*	*D. sandwicense* E. Mey	N993	Dr. K.S. McWhirter, U. of Sydney
Ds4	*D. sandwicense* E. Mey	CSL	Dr. J. Lotero C., I.C.A., Colombia
Ds5	*D. sandwicense* E. Mey	CWF	Dr. J. Lotero C., I.C.A., Colombia
Da1	*D. aparines*+(Link)D.C.	CPI 23189	Dr. K.S. McWhirter, U. of Sydney
Da2	*D. aparines*+(Link)D.C.	Q8382/56	Dr. K.S. McWhirter, U. of Sydney
Da3	*D. aparines*+(Link)D.C.	CPI 18010	Dr. K.S. McWhirter, U. of Sydney
Da4	*D. aparines*+(Link)D.C.	Desica-1	Dr. J. Lotero C., I.C.A., Colombia
Da5	*D. aparines*+(Link)D.C.	CPI 33814	Dr. B. Imrie, C.S.I.R.O., Brisbane
Dp	*D. pringlei* S.Watson	CPI 38722	Dr. K.S. McWhirter, U. of Sydney
Du	*D. uncinatum* (Jacq.) D.C.	CPI 8980	Dr. K.S. McWhirter, U. of Sydney
Dsp	?	Ecuador	Dr. B. Grof, C.I.A.T., Colombia
Dse	*D. sericophylum* (Schlecht)	CPI 33005	Dr. K.S. McWhirter, U. of Sydney

*((((Ds1 X Da1) X Ds1) X Ds1) x Ds1) - selfed 3 cycles until homozygosity for the spotted leaf character.

+also called *D. intortum* (Mill) Urb.

Five inbred lines of D. sandwicense (Ds1 to Ds5) were crossed in all possible combinations including reciprocal crosses to create a diallel cross. Although the inbred lines were not selected for the characters studied, the lines have been considered as a fixed sample of D. sandwicense lines. The six populations (P_1, P_2, F_1, F_2, BC_1, and BC_2) derived from a cross between two inbred lines were made for each of the following crosses: Ds1 x Da1, Ds2 x Dsp, and Ds2 x Ds5. The diallel cross and the three crosses between two inbred lines constituted the basic material to determine whether genetic variation among differing populations existed for symbiotic nitrogen fixation in Desmodium.

C. Experimental Procedures.

1. Crossing technique.

Two crossing methods have been used in Desmodium,. the emasculation method (Rotar et al., 1967) and the tripping method (McWhirter, pers. com.). The emasculation method is achieved by opening the flower bud the night before maturity and gently removing the anthers. The racemes with the emasculated flowers are then covered with a glassine bag containing a moist ball of cotton. During the morning hours, the flowers are pollinated with the desirable pollen using a sandpaper-tipped toothpick.

In the tripping method the mature flower is gently tripped and the stigma is examined with a 10X hand lens. Contaminated flowers are discarded. The remaining flowers are pollinated and following pod formation glassine bags are placed over the raceme to avoid seed contamination.

Rotar et al. (1967) found the emasculation technique to be superior but more difficult than the tripping method. They reported that the emasculation-wet cotton method produced 60% crossed seed from a 10% flower set compared to the tripping method which yielded 40% crossed seed from a 13% flower set. The authors only brushed the stigma in the tripping method which resulted in the low percentage crossed seed. Rotar et al. (1967) and Hutton (1960) both noted that crossing was most successful when the temperature was cool and the relative humidity was high. Rotar and Chow (1971) report that percentage pod set and the monthly average temperatures were negatively correlated ($r = -.99$, $df = 3$).

The crossing method used in this study was a modification of the tripping method. Each flower was grasped by the thumb and forefinger at the calyx and tripped by applying pressure at the base of the standard. Following tripping, the anthers and pollen were brushed away. Beginning with the top flowers, the stigmas of the tripped flowers were thoroughly washed with a 2.5% (by weight) sugar solution and immediately pollinated with the desired pollen. The pollen was either taken directly from the pollinating flower which was prepared by holding the flower with the thumb and forefinger at the calyx and gently removing the petals without disturbing the pollen or by tripping the pollinating flower directly onto a fingertip. The finger was dipped into alcohol between pollinations. Following pod formation the raceme was covered with a glassine bag to avoid seed contamination. Crosses were made at the Colombian Institute of Agriculture, Medellin, Colombia and at the University of Sydney, Sydney, Australia. Study of the progeny from some of these crosses showed that 95% of the F_1 crosses were hybrids for the spotted leaf character (Appendix A).

2. Bottle jars.

The bottle jars (Leonard jar assembly), for nitrogen-free growth containers, were constructed from various inexpensive and readily available materials. The bottle jar which conformed to the description of Vincent (1970) is illustrated in Figure 2. During this study, the bottle jar was modified to facilitate the removal of the plant roots intact for the acetylene reduction analysis. The modified bottle jar is illustrated in Figure 3. After filling the spirits bottle with sand and compacting the sand about the wick, the sand was wetted and the reservoir was filled with a nutrient solution composed of the following:

$CaHPO_4$	1.0 g
K_2HPO_4	.2 g
$MgSO_4 \cdot 7H_2O$	.2 g
NaCl	.2 g
$FeCl_3$	.1 g
Tap water	5.0 l

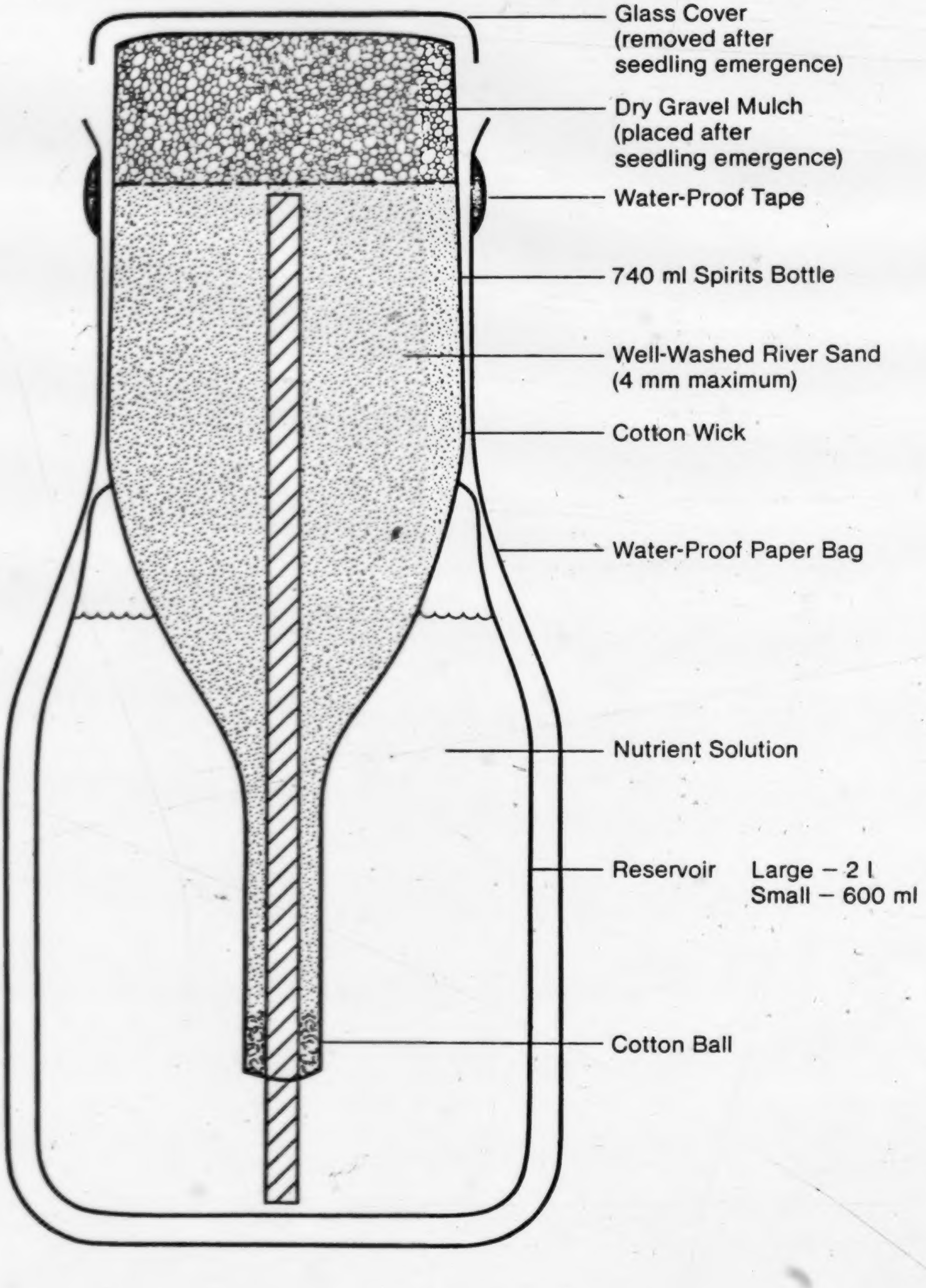

Glass Cover
(removed after
seedling emergence)
Dry Gravel Mulch
(placed after
seedling emergence)
Water-Proof Tape
740 ml Spirits Bottle
Well-Washed River Sand
(4 mm maximum)
Cotton Wick
Water-Proof Paper Bag
Nutrient Solution
Reservoir
Large – 2 l
Small – 600 ml
Cotton Ball

Figure 2. Initial bottle jar design (Vincent, 1970).

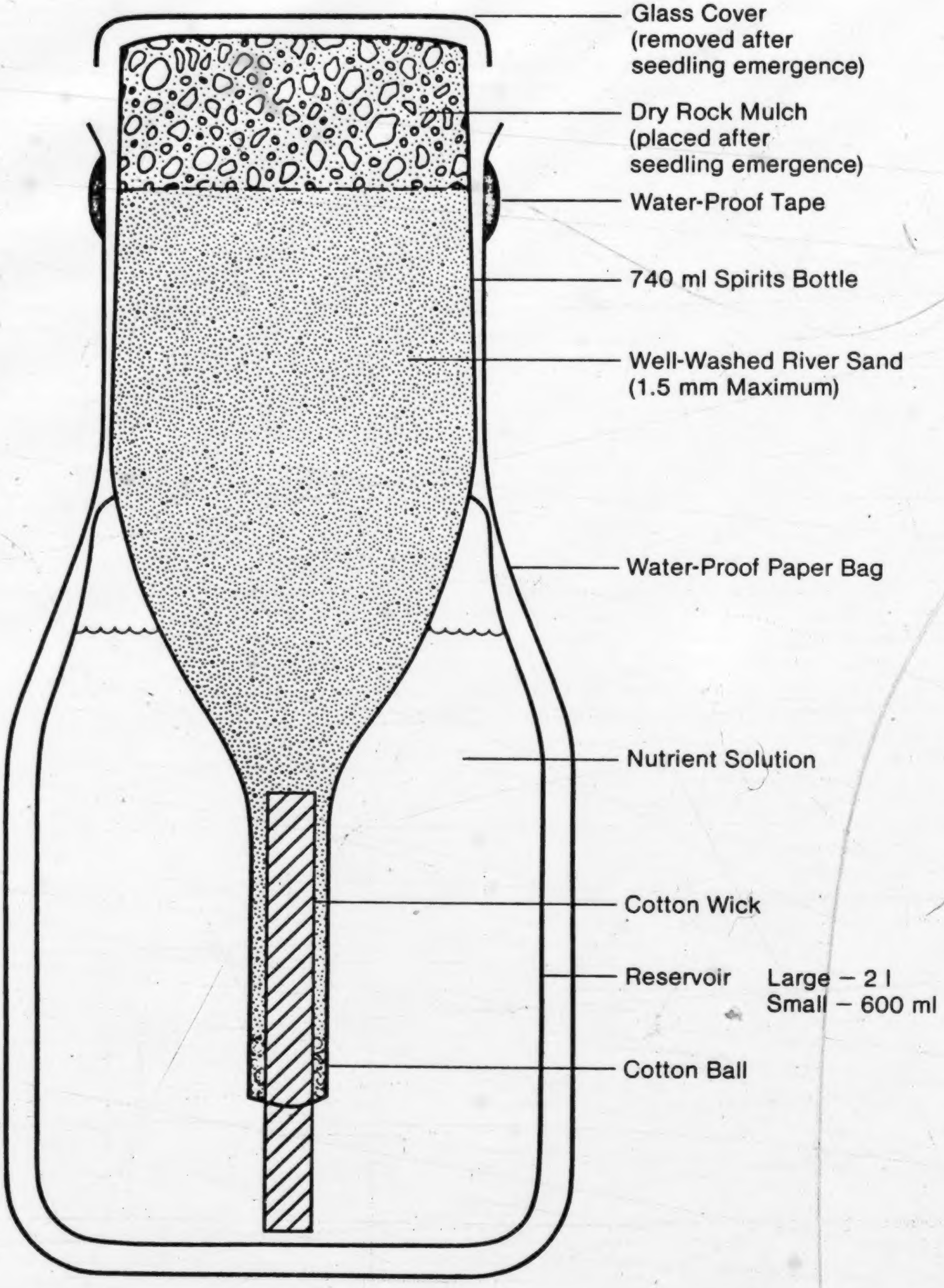

Glass Cover
(removed after
seedling emergence)
Dry Rock Mulch
(placed after
seedling emergence)
Water-Proof Tape
740 ml Spirits Bottle
Well-Washed River Sand
(1.5 mm Maximum)
Water-Proof Paper Bag
Nutrient Solution
Cotton Wick
Reservoir Large – 2 l
Small – 600 ml
Cotton Ball

Figure 3. Modified bottle jar design.

The bottle jar was covered with a water-proof paper bag which was secured with water-proof tape, capped with half of a petri-dish, and autoclaved for 2 hours at 15 p.s.i.. After cooling, the bottle jars were planted with surface sterlized seeds. The seeds were sterilized in an acidified 0.2% $HgCl_3$ solution (Vincent, 1970) and washed with six changes of sterile water. The testa of the seeds had been previously chipped to facilitate germination. Following germination and inoculation the bottle jars were covered with a pebble mulch (Vincent, 1970) and left undisturbed until harvest.

3. *Rhizobium* culture.

Stock cultures of the *Rhizobium* strains were maintained as agar slopes and stored at 2 C in McCartney bottles. Inoculum for the experiments was grown as broth cultures in 350 ml flats. The agar slopes and broth cultures were made from yeast-mannitol medium (Vincent, 1970) which consisted of the following ingredients:

K_2HPO_4	0.5	g
$MgSO_4.7H_2O$	0.2	g
NaCl	0.1	g
$CaCO_3$	3	g
Mannitol	10	g
Yeast water	100	ml
Distilled water	900	ml
Agar (when required)	15	g

Fifty ml of the above medium was placed in each flat and autoclaved at 15 p.s.i. (120 C) for 15 min. The sterile flats were stored at 2 C until required. Yeast water was freshly prepared by mixing 100 g of fresh compressed baker's yeast in one liter of cool water. The mixture was allowed to stand for 1 to 2 hours at room temperature before autoclaving for 50 min. The suspended material was allowed to settle and the clear supernatant was used as the yeast water.

Depending on the strain of Rhizobium, either ten to fourteen days prior to the inoculation time of the plants, a flat was removed from storage and inoculated with the desired strain using observed bacteriological precautions. The inoculated flat was placed in a growth cabinet at 26 C for the required time to facilitate the growth of the Rhizobium. For the inoculation of the experiments, a suspension of the Rhizobium was made by filling the flat with sterile water and shaking vigorously to disperse the Rhizobium. Five ml of this suspension was applied to the base of each plant 3 to 4 days following emergence, which was approximately 1 week after planting.

4. Quantitative variables.

(a) Acetylene reduction assay.

The acetylene reduction assay of Hardy et al. (1968) was used to assess the nitrogenase activity of the nodulated roots. Individual plants were harvested from the bottle jars and the root section from each plant was placed in a 13.5 ml vial which was plugged with an air tight seal. The vials were immediately placed on a vacuum system, refilled with argon and injected with 2.8 cc of oxygen and 3.5 cc of acetylene (ethylene free). After 60 min, a 200 microliter sample was removed from each vial and injected into a F-11 Perkin-Elmer gas chromatograph with a hydrogen flame ionization detector. The detector was attached to a Tohshin electron chart recorder. Nitrogen was used as the carrier gas and the column was a stainless steel tube of 30 cm in length and .32 cm in diameter and filled with Porapack T packing material. The column temperature was held at 60 C giving a retention time of 15 sec for ethylene and 30 sec for acetylene. At periodic intervals, duplicate samples were processed to check the accuracy of sampling. An acetylene reduction estimate (nanomolar(nM)/pl/hr) was calculated from peak height using the following equation:

$$\text{Acetylene reduced} = \frac{Es(Ef - Eo)}{As(Af - Ao)} \times Ac \times St$$

where Ef and Af were the final ethylene and acetylene peak heights, respectively, Eo and Ao were the origin values for ethylene and acetylene, and Es and As were the scales of measurement for ethylene and acetylene. Ac was the acetylene constant which was determined as the mean of the acetylene peak heights. St was the correction for a standard known sample of ethylene.

Acetylene reduction assay conducted in Experiment VI generally followed the procedure outlined above, except that a 35 ml vial was used with no argon. The ethylene peak was used as an estimate of acetylene reduced.

(b) Dry weight accumulation.

Dry weight accumulation data were collected for individual plants grown in bottle jar assemblies. The harvested plants were clipped at the cotyledonary node and the shoot portion was retained as the dry weight accumulation sample. The leaves dropped during the course of the experiment were collected and included in the sample. The samples were oven-dried at 105 C for 24 hours and left at room temperature for at least one week before weighing to the nearest milligram.

(c) Shoot length.

The shoot length was measured either *in situ* or on the harvested plants before oven drying with measurements being recorded to the nearest millimeter for the primary shoot length and the two secondary shoots originating at the cotyledonary node. Total shoot length was calculated by summing the primary and the two secondary shoot lengths.

D. Experimental designs and analyses.

1. Methodological studies.

(a) Selection for leaf size.

Prior to the commencement of the nitrogen fixation studies,

studies were conducted at the Colombian Agricultural Institute, Medellin, Colombia to become familiar with Desmodium growing in its natural habitat. A preliminary selection study was conducted with D. sandwicense for larger and smaller eighth node terminal leaflet area. Four hundred plants of a wild D. sandwicense population were selected as the base population. Several characters relating to the eighth node leaf were measured; namely, the 8th leaflet length and width, the petiole length, the internode length (between the 7th and 8th nodes), and the shoot length. From these characters the leaf area, the length/width ratio, the petiole/internode ratio, and the average internode length were computed.

Having defined the selection criterion (8th node terminal leaflet area) and the related characters, the selection was initiated by selecting five plants from the high and low 'tails' of selection criterion distribution and randomly mating the plants within each line. For the first cycle of selection, duplicate data for leaf size were recorded for the tenth leaf and were included to determine their response to a selection pressure applied to the eighth node terminal leaflet. One hundred plants were grown for each selection cycle and selecting five plants from the 'tails' of the selection criterion distribution. The data for all characters for cycles 1 and 2 were analysed with a one-way analysis of variance to determine whether the characters were responding to selection.

(b) Growth patterns.

To determine whether the bottle jar was an effective container for the study of nitrogen fixation in _Desmodium_, a study was initiated to observe the growth of _Desmodium_ in a bottle jar. The fifteen diallel genotypes were planted, two plants of one genotype per bottle jar, along with five (inbred parents) uninoculated controls to determine a mean growth curve. The experiment was conducted during the winter season and the primary shoot length was recorded every twenty days for 100 days. From the mean growth curve, a sampling point for the nitrogen fixation studies would be determined.

(c) Linearity of acetylene reduction.

Hardy _et_ _al_. (1968) reported that following injection of acetylene into a vial with nodulated soybean roots, acetylene was reduced to ethylene at a linear rate until approximately 90 minutes following injection. To verify that acetylene reduction by nodulated _Desmodium_ roots occurred at a linear rate, four _Desmodium_ species (Ds1, Da1, Dp, Du; Table 2) of plant breeding interest were inoculated with strain A and grown for 8 weeks. Two plants for each line were bulk harvested and prepared for the acetylene reduction assay. Samples, 50 microlitres each, for each vial were extracted and processed at 10 min. intervals.

The five diallel lines were also prepared for the acetylene reduction assay and a 50 microlitre sample was measured every 10 min. to determine if fixation was linear.

(d) Bottle jar design.

A test of the bottle jar design was initiated by screening river sand into the following four sizes:

(a) < 4.0 mm (equivalent to that of Vincent (1970))

(b) 1.0 - 3.0 mm

(c) 1.0 - 1.5 mm

(d) < 1.0 mm

The sand sizes a to c were placed in the initial bottle jar design (Figure 2) and in the modified design (Figure 3) for size d. The sand in all bottle jars was compacted about the wick to ensure capillary movement of the nutrient solution. The bottle jars were arranged into a randomized block experiment with six blocks, planted with the Ds1 line and inoculated with strain B. Three seeds were planted in each bottle jar and after the rock mulch was applied, each cell was thinned to the two most vigorous plants. The experiment was harvested after eight weeks and the data for dry weight and shoot length were recorded for each cell as: (a) the mean of the two plants, and (b) the difference of the two plants in the same bottle (within-cell difference). The cell mean data were analysed with a two-way analysis of variance to determine significant differences for dry weight accumulation among sand sizes.

2. Strain-specific ineffective nodulation interaction.

Strain-specific ineffective nodulation interactions between some lines of *D. aparines* and strain B were found in this study. To determine the mode of inheritance of these interactions, *D. sandwicense* (Ds1) X *D. aparines* (Da1 and Da2) cross plants were scored for green or yellow-chlorotic color in the F_2 and backcross (to Ds1) generations to determine the segregation ratios. The scored results were tested with the chi-square goodness of fit to an expected segregation ratio.

Two hundred and fifty F_2 seeds, 150 backcross seeds and 10 seeds each for parental controls were planted in bottle jars and inoculated with 5 ml of strain B to each seed. At eight weeks the plants were scored for green or yellow color and a random sample of seventy ineffectively nodulated F_2 plants were removed from the bottle jars and inspected for nodulation characteristics. Following inspection these plants were planted in sand and fertilized with a source of nitrogen to determine if nitrogen was, in fact, the limiting criterion.

Since some lines of *D. aparines* display the spotted leaf character, all the plants were also scored for this character and tested for a chi-square goodness of fit to an expected segregation ratio. Joint segregation tests were determined for ineffective/effective nodulation and spotted/plain leaves.

3. Experiment I.

The diallel cross was arranged in Experiment I as a randomized block design with four blocks (replicates) and fifteen bottle jars per block. The bottle jars were randomly assigned the fifteen diallel genotypes and were randomized on a 30 cm grid within each block. Similar bottle jars (original design, Figure 2) were used within each block with blocks 1 and 4 having similar bottle shapes while blocks 2 and 3 were different. Each bottle jar was planted with two surface sterilized seeds and each plant was inoculated with a 5 ml suspension of strain B following emergence. Blocks were planted at differing planting dates due to space limitations and to facilitate the acetylene reduction analysis. Experiment I was conducted during the winter season at the University of Sydney; therefore, the average glasshouse temperature was near 21 C.

Each block was harvested after a growth period of 10 weeks and data relating to shoot length, dry weight accumulation and the acetylene reduction assay were recorded as the mean of the two plants in each bottle jar.

Although the inbred lines were not selected for the characters studied, the lines have been considered as a fixed sample of *D. sandwicense* lines. The data were analysed under the assumptions of the model I, method 2 (Griffing, 1956) and Analysis II (Gardner and Eberhart, 1966) diallel design to establish the combining ability effects (Griffing, 1956) and genetic constants (Gardner and Eberhart, 1966), respectively.

4. Experiment II.

The Experiment II diallel cross was arranged as a randomized block design with four blocks (replicates) and fifteen bottle jars per block. The bottle jars were randomly assigned the fifteen diallel genotypes and were randomized on a 30 cm grid within each block. Similar bottle jars (original design, Figure 2) were used within each block with blocks 1 and 2 having one bottle shape, whereas blocks 3 and 4 had another. Each bottle jar was planted with two surface sterilized seeds and each plant was inoculated with a 5 ml suspension of strain A following emergence. Blocks were planted at different planting dates due to space limitations and to facilitate the acetylene reduction analysis. Experiment II was conducted during the spring season at the University of Sydney; therefore, the average glasshouse temperature was near 24 C.

Each block was harvested after a growth period of 10 weeks and data relating to dry weight accumulation and the acetylene reduction assay were recorded for each plant. The data were analysed under the assumptions of the model I, method 2 (Griffing, 1956) and Analysis II (Gardner and Eberhart, 1966) diallel design to establish the combining ability effects and genetic constants.

5. Experiment III.

Experiment III was grown as a factorial experiment with two strains (A and B), two blocks for each strain, and fifteen bottle jars per block with three plants for one genotype planted in each bottle jar. The fifteen diallel genotypes were randomly assigned to bottle jars which were randomized on a 30 cm grid within each block. Each block was randomly assigned a bench location and blocks inoculated with different strains were separated on the bench to avoid contamination. The total experiment was planted on one day at the University of Sydney during the summer season. The mean glasshouse temperature for this experiment was approximately 26 C.

Similar types of bottle jars (modified design, Figure 3) were autoclaved in two groups such that autoclaving has been confounded with blocks.

During the early juvenile leaf stage, Experiment III was invaded by insect larvae. Since leaf loss resulted, the experiment was sprayed with an insecticide and each plant was clipped back to the cotyledonary node. After 10 weeks, all plants were harvested and measurements for the acetylene reduction assay were taken for strain A on one day and for strain B the following day. One block for each strain was analysed during the late morning with the other block analysed during the early afternoon. Dry weight accumulation were also recorded for the shoot portion of each plant. Due to the death of several plants, the two plants with the largest dry weight in each bottle jar were retained for diallel analysis. The data were analysed under the assumptions of the model I, method 2 (Griffing, 1956) and Analysis II (Gardner and Eberhart, 1966) diallel design to establish the combining ability effects and genetic constants.

6. Combined analysis.

The same diallel cross was used in the preceding diallel experiments; therefore, the data may be pooled for a comprehensive analysis. Data for individual plants were available for block 4 in Experiment I and all blocks in Experiments II and III giving nine blocks in total. Three of these blocks were inoculated with strain B while the remaining six were noculated with strain A. Since strains have been considered as environmental effects for this study, strains have been pooled with blocks for the combined analysis. The data were analysed under the assumptions of the model I, method 2 (Griffing, 1956) and Analysis II (Gardner and Eberhart, 1966) diallel design to establish the combining ability effects and genetic constants.

7. Experiment IV.

A cross between the two inbred lines, Ds1 x Da1 (Table 2) was made creating the six populations: (P_1(Ds1), P_2(Da1), F_1, F_2, BC_1 (P_1 x F_1), and BC_2 (P_2 x F_1). Experiment IV was grown during the spring season at the University of Sydney as a randomized block design with seven blocks and twelve bottle jars per block. All bottle jars were of the original design (Figure 2) and blocks 1 to 5 had 600 ml reservoirs (Figure 2) while blocks 6 and 7 had 2 l reservoirs. The six populations were allotted to the twelve bottle jars such that the P_1, P_2 and F_1 populations received one bottle jar each, the F_2 population received five, and the backcross populations received two each. The bottle jars were randomized within each block and all blocks were planted on the same day with surface sterilized seeds. After emergence, each plant was inoculated with 5 ml of a suspension of strain A. The experiment was harvested after seven weeks growth with dry weight accumulation and the acetylene reduction assay being recorded for each plant. The analysis of variance of population means (Hayman, 1958) was conducted as a two-way analysis of variance with the population means within each block constituting the data which was analysed as outlined in Section E2. Although Hayman did not partition epistatic effects, a study was initiated to investigate the feasibility of such a partition (see Results and Discussion sec. A5).

8. Experiment V.

The six populations of the Ds2 x Dsp (Table 2) cross were grown in Experiment V as a randomized block design with five blocks and fifteen bottle jars per block. All bottle jars were of the modified design (Figure 3) and all had 600 ml reservoirs (Figure 3). The six populations were allotted to the fifteen bottle jars such that the P_1, P_2 and F_1 populations received one bottle jar each, the F_2 population received six, and the backcross populations received three each. The bottle jars were randomized within each block and all blocks were planted on the same day. After emergance of each plant was inoculated with a 5 ml suspension of strain B. The experiment was harvested after ten weeks growth.

Dry weight accumulation and the acetylene reduction assay were recorded for each plant and the analysis of variance of population means (Hayman, 1958) was conducted as a two-way analysis of variance with the population means within each block constituting the data which were analysed as outlined in Section E2.

9. Experiment VI.

Experiment VI consisted of the six populations from the cross between the inbred lines Ds2 and Ds5 (Table 2) and was grown in a growth chamber at the University of Alberta. Three plants of one population were grown in a ten cm pot and inoculated at planting with five ml of a suspension of strain A. The pots were arranged into four blocks; two of which had sand of very fine (.15mm) particle size, one with fine (.25mm) sand, while the remaining block was a 50% mixture of each sand size. Within each block the populations were allotted such that 5 pots each were allotted to the P_1 (Ds2), P_2 (Ds5), and F_1 populations while 20 pots were allotted to the the F_2 population and 10 pots each to the backcross populations. All pots were wetted with the nutrient solution prior to planting. The experiment was maintained at a temperature of 23 C and harvested after 11 weeks. Data recorded for each plant were the acetylene reduction assay, shoot fresh and dry weight, root fresh weight, the cotyledon length and width (first node), and the width of the juvenile leaf (second node) since the juvenile leaf is nearly circular. The data were analysed on an individual plant basis as outlined in Section E2.

E. Genetical analyses.

1. Diallel cross.

A diallel cross is a set of all possible crosses among a set of inbred lines and the diallel table is a square matrix of dimension equal to the number of parents. The main diagonal represents the inbred lines while the data above and below the main diagonal represents the F_1 and reciprocal F_1 crosses, respectively. In the absence of reciprocal effects the matrix is symmetrical. A diallel table may be formed by four methods from the components of the square matrix (Griffing, 1956).

The diallel analysis of variance of the data in the diallel table has been reviewed and discussed by several workers (Sprague and Tatum, 1942; Hayman, 1954; Griffing, 1956; Cockerham, 1963; Gardner and Eberhart, 1966) and the relationship among the four methods and the analyses for each method reviewed by Baker, 1978.

The interpretation of the diallel analysis of variance depends upon the model under investigation. Griffing (1956) reported four differing models underlying the analysis of variance. The models are:

Model I. Fixed treatment and block effects

Model II. Random treatment and block effects

Model A. Random treatment and fixed block effects

Model B. Fixed treatment and random block effects.

The basic difference between these models relates to the difference in the interpretation of the fixed and random treatment (genotype) effects. The fixed model assumes that the inbred lines and their F_1 crosses constitute the total population about which inferences are to be made and estimates combining ability effects and variances for the inbred lines. The random model assumes that the inbred lines are a random sample from an ancestral population about which inferences are to be made. The random model estimates the genetic and non-genetic components of the population variance.

Hayman (1954) presented several assumptions underlying a diallel analysis which must be satisfied before the conclusions may be considered reliable. These assumptions are:

(a) that the parents are homozygous,

(b) there is diploid segregation,

(c) there are no reciprocal effects,

(d) there is no multiple allelism,

(e) the genes are independently distributed, and

(f) there is independent action of non-allelic genes (epistatic effects).

Griffing (1956) reported that the independent action of the non-allelic genes was the assumption of major importance. The diallel cross among inbred lines of D. sandwicense used in this study satisfied assumptions (a) and (b) while assumptions (c) and (d) were considered to be of minor importance. Assumptions (e) and (f) concerning linkage and epistatic effects, respectively, were of major concern in the present investigation since Desmodium has never been tested for such effects.

The analysis of the acetylene reduction assay, the dry weight accumulation characters and other characters were based on a Model I, method 2 diallel analysis as outlined by Griffing (1956) and modified to include Analysis II of Gardner and Eberhart (1966). The Model I, method 2 diallel analysis used in this study implies a statistical rather than a genetic interpretation (Baker, 1978) to partition the genetic component sum of squares and to estimate genetic constants (Gardner and Eberhart,1966) and combining ability effects (Griffing, 1956). A listing of the Fortran computer program employed in this study for the diallel analysis of the data is presented in Appendix B.

2. Cross between two inbred lines.

An analysis of a cross between two inbred lines as outlined by Mather and Jinks (1971) may consist of six or more populations. The six populations of the minimum design are: parent 1 (P_1), parent 2 (P_2), the initial cross (F_1), the segregating generation (F_2), and the backcrosses of the F_1 to both parents (B_1 and B_2). For this fixed model, estimates of the type of gene action may be obtained from first degree statistics (Mather and Jinks, 1971).

Estimates of the magnitude and significance of the genetic parameters were calculated from the population means with the formulae as presented by Mather and Jinks (1971). The equations for the calculation of the genetic parameters are:

$m = .5\bar{P}_1 + .5\bar{P}_2 + 4\bar{F}_2 - 2\bar{B}_1 - 2\bar{B}_2$

$a = .5\bar{P}_1 - .5\bar{P}_2 + \bar{B}_1 - \bar{B}_2$

$d = 1.5\bar{P}_1 - 1.5\bar{P}_2 - \bar{F}_1 - 8\bar{F}_2 + 6\bar{B}_1 + 6\bar{B}_2$

$aa = - 4\bar{F}_2 + 2\bar{B}_1 + 2\bar{B}_2$

$ad = -\bar{P}_1 + \bar{P}_2 + 2\bar{B}_1 - 2\bar{B}_2$

$dd = \bar{P}_1 + \bar{P}_2 + 2\bar{F}_1 + 4\bar{F}_2 - 4\bar{B}_1 - 4\bar{B}_2$

where

m = F_2 mean

a = additive effects

d = dominance effects

aa = additive X additive interaction

ad = additive X dominance interaction

dd = dominance X dominance interaction.

These equations allow for an estimate of the magnitude of the parameters to be made with the significance of each being tested by the t-test of the estimate to its standard deviation (Mather and Jinks, 1971). The variance of each parameter is determined from the variances of the six populations. For example, an estimate of the variance of d is obtained from the following equation:

$$Vd = 2.25VP_1 + 2.25VP_2 + VF_1 + 64VF_2 + 36VB_1 + 36VB_2$$

where Vd = the variance of the dominance deviations, VP_1 = the variance of P_1, VP_2 = the variance of P_2, etc.

An alternate analysis of population means is the analysis of variance of the population means (Hayman, 1958; Voigt _et al._, 1966). To estimate the genetic parameters of the model a coded matrix was constructed (Mather and Jinks, 1971). The coded matrix is as follows:

Mean	a	d	aa	ad	dd
$\bar{P}_1$	1	0	1	0	0
$\bar{P}_2$	-1	0	1	0	0
$\bar{F}_1$	0	1	0	0	1
$\bar{F}_2$	0	1/2	0	0	1/4
$\bar{B}_1$	1/2	1/2	1/4	1/4	1/4
$\bar{B}_2$	-1/2	1/2	1/4	-1/4	1/4

The matrix may be weighted by dividing each row by the standard error of the population mean. The coded matrix was subjected to a stepwise least squares analysis (Draper and Smith, 1966) which has been restricted in this investigation. The genetic parameters of the model were estimated on the assumption that within locus effects are of more importance than between loci interactions in a genetic model; i.e. a simple model. Therefore the variance among the population means was first analysed for additive and dominance effects before estimating the interaction parameters (see Results and Discussion, Section A5). The significance of the parameter mean squares was tested by the F-ratio with the error term from the analysis of variance of population means as the denominator (Voigt *et al.*, 1966).

Properly coding the genotypes, replications, etc. prior to date entry allows the usage of the SPSS statistical analysis package (Nie, *et al.*, 1975) for analysing the data for a cross between two inbred lines. SPSS is particularily suited to the recoding of the data or the creation of new variables to assist in the complete analysis and interpretation of the data. The partitioning of the population sum of squares into its genetic components is not available in SPSS; however, an interactive Fortran computer program, listed in Appendix C, was developed for this analysis. Program HAYMAN follows the analytical procedure for the analysis of variance of population means as defined by Hayman (1958) and earlier discussed. HAYMAN is suited for genetic analysis of any type of statistical design whether a one-way or a multi-factorial analysis of variance or covariance since the population sum of squares is partitioned into genetic components by the correlation form of the stepwise regression analysis. This regression analysis is independent of scale or design (Draper and Smith, 1966).

(3) Principal component transformations.

Total nitrogen fixation has been estimated in this study by many measures and correlations among the measures would be expected. When variables are highly correlated the communal variation within the correlation matrix can be summarized with a principal components transformation (Winer, 1971) to construct "variables" which estimate the variation along the axes of principal variation. This transformation is also called principal components analysis (Overall and Klett, 1972) where the primary emphasis is to define "composite or factor-score variates that have certain desirable statistical properties". Principal components analysis may be divided into three stages of analysis. Firstly the analysis begins with the calculation of a correlation or a covariance matrix. Then the correlation or covariance matrix is mathematically reduced to eigenvectors (factor loadings) and eigenvalues (the amount of variance accounted by each eigenvector). Generally a correlation matrix with unity (total variances) along the main diagonal is structured because this matrix is scale independent (Overall and Klett, 1972). Finally, factor score coefficients are calculated from the factor loading matrix with a straight-forward mathematical procedure analogous to a regression of an unknown dependent variable on the original variables. A composite variable is a linear combination of factor score coefficients of the variables. The first composite variable accounts for greatest amount of common variation in the data. The second composite variable is orthogonal to the first and accounts for the next largest portion of the common residual variation. The remaining composite variable accounts for the remaining variation. Principal components analysis, being primarily concerned with construction of composite variable scores, normalizes the eigenvectors by converting the eigenvector matrix to one of correlations among the original variables and the derived factors. Such a normalization is of interest in determining what the principal axes represent. From the normalized eigenvectors, composite variables are defined which maximize existing differences among individual cases.

Principal components analysis has been used in agronomy by Denis and Adams (1978) to relate plant variables to yield of Phaseolus vulgaris L. (common bean) and by Hussaini, et al. (1977) to define major groupings within the world collection of Eleusine coracana L. (finger millet). However, principal components analysis has not been employed as a method of analysing differences among the population means. Since the principal axis of variation is expected to be nitrogen fixation, it would be pertinent to define this axis and investigate its variation.

The principal axis of variation in this study was scaled to a distribution with a mean and standard deviation of 1/3 that of the acetylene reduction assay. This scale was used because nitrogenase theoretically reduces three times the amount of acetylene than N_2 (Burris, 1974). Following scaling, the composite N_2 fixation estimate was treated as another character for the analyses. The interpretation of the principal axis of variation will be restricted to the investigation of the differences among population means rather than attempting to estimate the amount of nitrogen fixed.

RESULTS AND DISCUSSION

A. Methodology

To achieve the ultimate goal of lines or hybrids with greater total nitrogen fixation it is necessary to attain an understanding of the genetic mechanisms operating in the host. In this study, the area of genetic mechanisms of nitrogen fixation has been divided into three levels (Table 1). In this generalized section of methodology several studies were undertaken both initially and during experimentation to establish or to investigate various aspects of methodology.

1. Selection for leaf size.

A preliminary study was initiated in *Desmodium* to become familiar with this species growing in its natural habitat. This study involved applying a selection pressure to the eighth node terminal leaflet area to determine its response to selection. The frequency distribution of the eighth node terminal leaflet sizes for the base population are presented in Figure 4. The distribution indicated that considerable variation was present in the wild population. Principal components analysis of the base population characters suggested that most characters were related to one factor i.e. a leaf size factor (Factor 1, Table 3) while the ratio characters are individually related to other factors.

Data collected for the tenth node leaf for a Dsp (Table

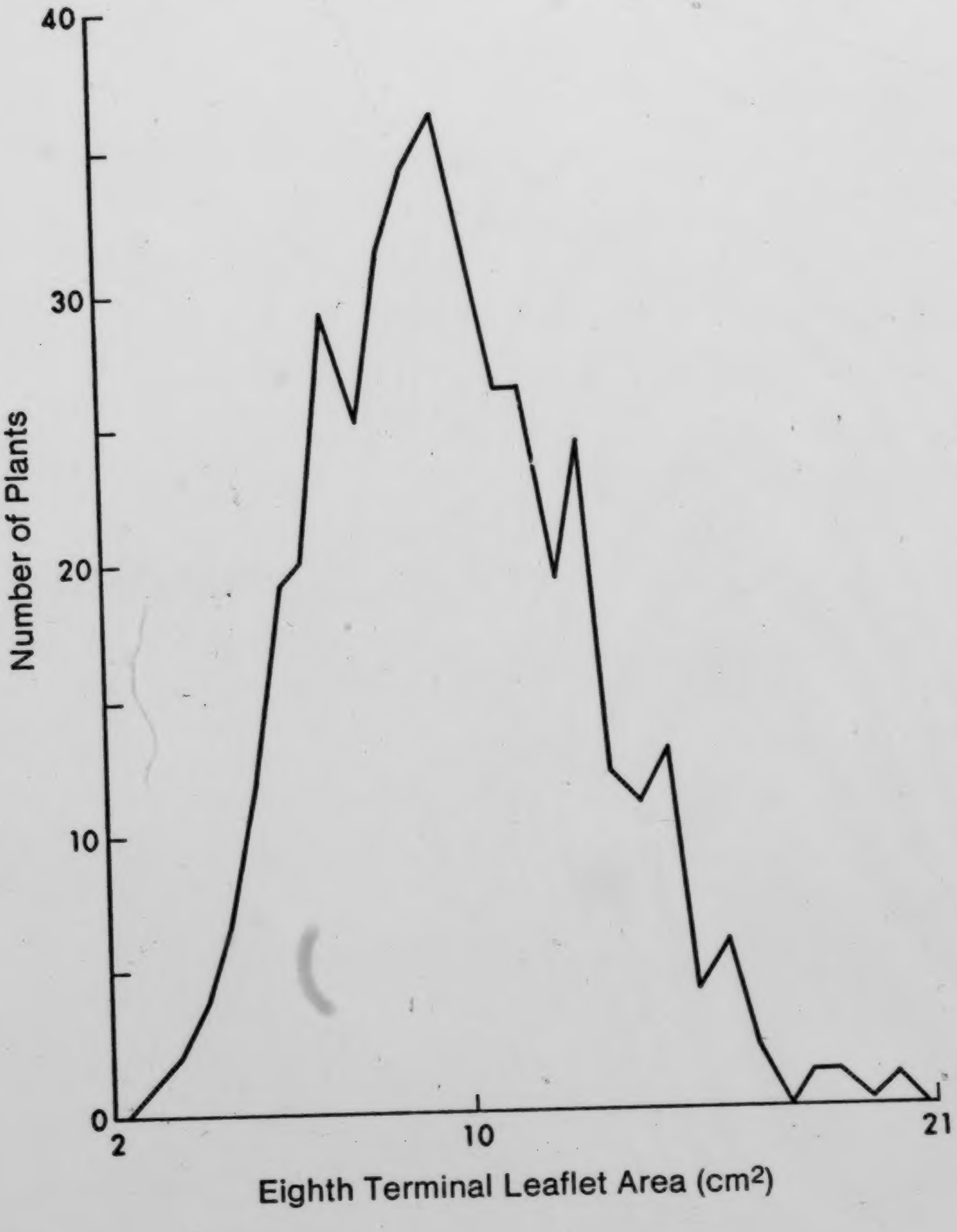


Figure 4. Frequency distribution of the eighth node terminal leaflet area for the base population.

Table 3. Eigenvectors for factors from the base population.

	Factor		
Character	1	2	3
Terminal leaflet length(8*)	0.94	0.07	0.01
Terminal leaflet width(8)	0.91	0.05	-.34
Terminal leaflet area(8)	0.94	0.06	-.17
Length/width ratio(8)	-.04	0.02	0.95
Petiole length(8)	0.85	0.28	0.11
Internode length(7-8)	0.74	-.54	0.11
Petiole/internode(8)	0.10	0.96	0.04
Shoot length	0.81	-.33	0.25
Average internode length	0.81	-.41	0.25

*node number.

2) population indicated that leaf measurements were related to one factor while plant weights related to another (Table 4). Ratio characters again were related each to a single factor.

Principal components analysis of the cycle 1 data still suggested leaf size characters were related to the first factor while ratio characters were related to another (Table 5). The means and standard deviations for each character for cycle 1 are presented in Table 6. The base population was found to have a significantly larger eighth terminal leaflet area than either the high or low selection lines with the exception of the ratio characters where the reverse was true. Means and standard deviations for the factor score variables indicated the same patterns as the selection criterion (Table 7).

Cycle 2 data also indicated the same pattern between the characters and the factors (Table 8). The response to selection by the high line was found to be negative for cycle 1; however, the high line mean was found to be significantly larger than the base mean suggesting a response to selection (Table 9). The low line mean was found to be significantly lower than the base mean while the factor score means also indicated that the response to selection away from the base population was significant (Table 10). The analyses suggested that the leaf size characters were highly related as indicated by their factor loadings to factor 1 and similarily with which they

Table 4. Eigenvectors for factors from the Dsp population.

Character	Factor			
	1	2	3	4
Terminal leaflet length(10*)	-.04	0.94	-.07	0.28
Terminal leaflet length(10)	-.06	0.97	-.07	-.12
Terminal leaflet area(10)	-.06	0.97	-.10	0.10
Length/width ratio(10)	0.05	0.18	0.00	0.96
Petiole length (10)	0.14	0.88	0.15	0.11
Internode length (9-10)	-.06	0.73	-.57	0.07
Petiole/internode ratio(10)	0.19	-.06	0.93	0.01
Shoot length	0.91	0.03	-.21	0.17
SQRT(no. of nodes)	0.76	-.36	0.16	-.10
Average internode	0.73	0.26	-.32	0.28
SQRT(no. of tillers)	0.76	-.12	0.40	.00
Shoot weight	0.95	0.01	0.17	-.03
Leaf weight	0.88	-.07	0.19	0.01
Total weight	0.93	-.04	0.19	0.00
Leaf/shoot ratio	-.72	-.14	0.01	0.11

*node number.
SQRT - square root.

Table 5. Eigenvectors for factors from cycle 1.

Character	Factor 1	2	3	4
Terminal leaflet length(8*)	0.88	-.27	-.01	0.22
Terminal leaflet width(8)	0.89	-.26	-.04	-.20
Terminal leaflet area(8)	0.91	-.28	-.04	0.02
Length/width ratio(8)	-.04	-.03	0.07	0.84
Petiole length (8)	0.74	0.42	0.18	0.11
Internode length (7-8)	0.36	-.76	0.28	0.10
Petiole/internode ratio(8)	0.14	0.92	-.15	-.03
Terminal leaflet length(10)	0.91	0.17	0.12	0.17
Terminal leaflet width(10)	0.88	0.20	0.05	-.31
Terminal leaflet area(10)	0.93	0.20	0.08	-.07
Length/width ratio(10)	0.01	-.05	0.10	0.85
Petiole length(10)	0.56	0.33	-.39	-.01
Internode length(9-10)	0.46	0.02	0.75	0.09
Petiole/internode ratio(10)	0.10	0.27	-.93	-.07

*node number.

Table 6. Means and standard deviations from a one-way analysis of variance of the selection variables for cycle 1.

		Eighth node						
		Terminal leaflet						
Selection line	n	Length (cm)	Width (cm)	Area* (cm^2)	Length/ width ratio	Petiole Length (cm)	Internode length (cm)	Petiole/ internode ratio
High	100	5.0±.39a+	3.8±.29b	19.2±2.8b	1.3±.06a	6.6±.81a	5.7±0.74b	1.2±.20a
Base	100	5.0±.47a	4.0±.33a	20.2±3.3a	1.1±.07b	6.4±.74b	6.4±1.39a	1.0±.25b
Low	100	4.4±.32b	3.5±.29c	15.4±2.3c	1.3±.06a	5.8±.53c	5.6±0.85b	1.1±.17b

*selection criterion.

+means followed by the same letter are not significantly different at the 5% level for the new multiple range test.

Table 6 (cont). Means and standard deviations from a one-way analysis of variance of the selection variables for cycle 1.

		Tenth node						
		Terminal leaflet						
Selection line	n	Length (cm)	Width (cm)	Area (cm^2)	Length/ width ratio	Petiole length (cm)	Internode length (cm)	Petiole/ internode ratio
High	100	6.0±.49b+	4.5±.38a	27.0±4.3a	1.3±.07b	5.6±1.30b	8.7±1.35a	0.65±.18c
Base	100	6.1±.50a	4.4±.34a	27.3±3.9a	1.4±.08a	6.0±1.05a	8.8±1.71a	0.70±.16b
Low	100	5.4±.38c	4.0±.38b	21.5±3.3b	1.3±.08b	5.6±0.72b	7.6±1.07b	0.74±.11a

+means followed by the same letter are not significantly different at the 5% level for the new multiple range test.

Table 7. Means and standard deviations from a one-way analysis of variance of the factors for cycle 1.

		Factor			
Selection line	n	1	2	3	4
High	100	0.28±.88b*	0.36±0.89a	0.34±1.14a	-0.09±0.92a
Base	100	0.53±.81a	-0.36±1.19c	-0.02±1.02b	0.12±1.10a
Low	100	-0.82±.71c	-0.02±0.75b	-0.33±0.69c	-0.03±0.96a

*means followed by the same letter are not significantly different at the 5% level for the new multiple range test.

Table 8. Eigenvectors for factors from cycle 2.

	Factor		
Character	1	2	3
Terminal leaflet length(8*)	0.95	-.12	0.05
Terminal leaflet length(8)	0.92	-.09	-.24
Terminal leaflet area(8)	0.94	-.12	-.10
Length/width ratio(8)	-.17	-.03	0.84
Petiole length (8)	0.91	-.06	-.11
Internode length (7-8)	0.56	-.75	-.11
Petiole/internode ratio(8)	0.16	0.89	0.04
Terminal leaflet length(10)	0.96	0.02	0.06
Terminal leaflet width(10)	0.93	0.01	-.30
Terminal leaflet area(10)	0.96	0.00	-.13
Length/width ratio(10)	-.14	0.05	0.86
Petiole length (10)	0.84	0.21	-.20
Internode length (9-10)	0.64	-.63	-.10
Petiole/internode ratio(10)	0.11	0.90	-.11

*node number.

Table 9. Means and standard deviations from a one-way analysis of variance of the selection variables for cycle 2.

		Eighth node						
		Terminal leaflet						
Selection line	n	Length (cm)	Width (cm)	Area* (cm^2)	Length/ width ratio	Petiole length (cm)	Internode length (cm)	Petiole/ internode ratio
High	95	4.5±.66a[+]	3.4±.53a	15.6±4.4a	1.3±.07b	6.3±1.06a	5.5±0.99a	1.2±.27b
Base	99	3.9±.64b	3.0±.49b	12.2±3.7b	1.3±.08b	5.2±0.97b	4.1±1.07b	1.3±.34a
Low	95	3.4±.46c	2.5±.38c	8.6±2.2c	1.4±.10a	4.6±0.77c	3.6±0.71c	1.3±.22a

*selection criterion.

+means followed by the same letter are not significantly different at the 5% level for the new multiple range test.

Table 9 (cont). Means and standard deviations from a one-way analysis of variance of the selection variables for cycle 2.

		tenth node						
		terminal leaflet						
Selection line	n	length (cm)	width (cm)	area (cm^2)	length/ width ratio	petiole length (cm)	internode length (cm)	petiole/ internode ratio
High	95	4.8±.82a+	3.9±.65a	20.8±6.2a	1.4±.09b	6.1±1.31a	8.4±1.44a	.73±.17b
Base	99	4.8±.66a	3.5±.48b	17.1±4.3b	1.4±.11b	5.2±0.99b	6.6±1.56b	.83±.22a
Low	95	4.2±.54b	2.9±.36c	12.0±2.8c	1.5±.13a	4.5±0.81c	6.0±1.20c	.77±.15b

+means followed by the same letter are not significantly different at the 5% level for the new multiple range test.

Table 10. Means and standard deviations from a one-way analysis of variance of the factors for cycle 2.

		Factor		
Selection line	n	1	2	3
High	95	0.74±.98a*	-0.47±0.97b	-0.20±0.80b
Base	99	0.00±.79b	0.28±1.12a	-0.23±0.97b
Low	95	-0.74±.59c	0.17±0.70a	0.44±1.06a

*means followed by the same letter are not significantly different at the 5% level for the new multiple range test.

responded to a selection pressure upon one of them. The selection response for the eighth node terminal leaflet and the first principal factor over two cycles has been summarized in Figure 5.

2. Growth patterns.

An initial study was conducted with the crosses among *D. sandwicense* lines inoculated with strain B to observe the growth of *Desmodium* in a bottle jar and to observe the general growth pattern over a period of time. The mean growth patterns of the inoculated and uninoculated plants are presented in Figure 6. During the winter season, the transition from lag to log growth phase occurred at approximately the 10th week for the inoculated plants. Since this transition occurred later in the winter season, the 10th week was chosen as an appropriate sampling time for a study of the relationship between dry weight accumulation and the acetylene reduction assay. The uninoculated controls were yellow and chlorotic and their height did not increase beyond the height at juvenile stage. This chlorotic condition suggested that the bottle jar was an effective assembly for the study of symbiotic nitrogen fixation in *Desmodium*.

Diallel tables for several characters measured on the inoculated plants of the above study were constructed and partioned into genetic component mean squares which are expressed as a percentage of the genotype mean square and

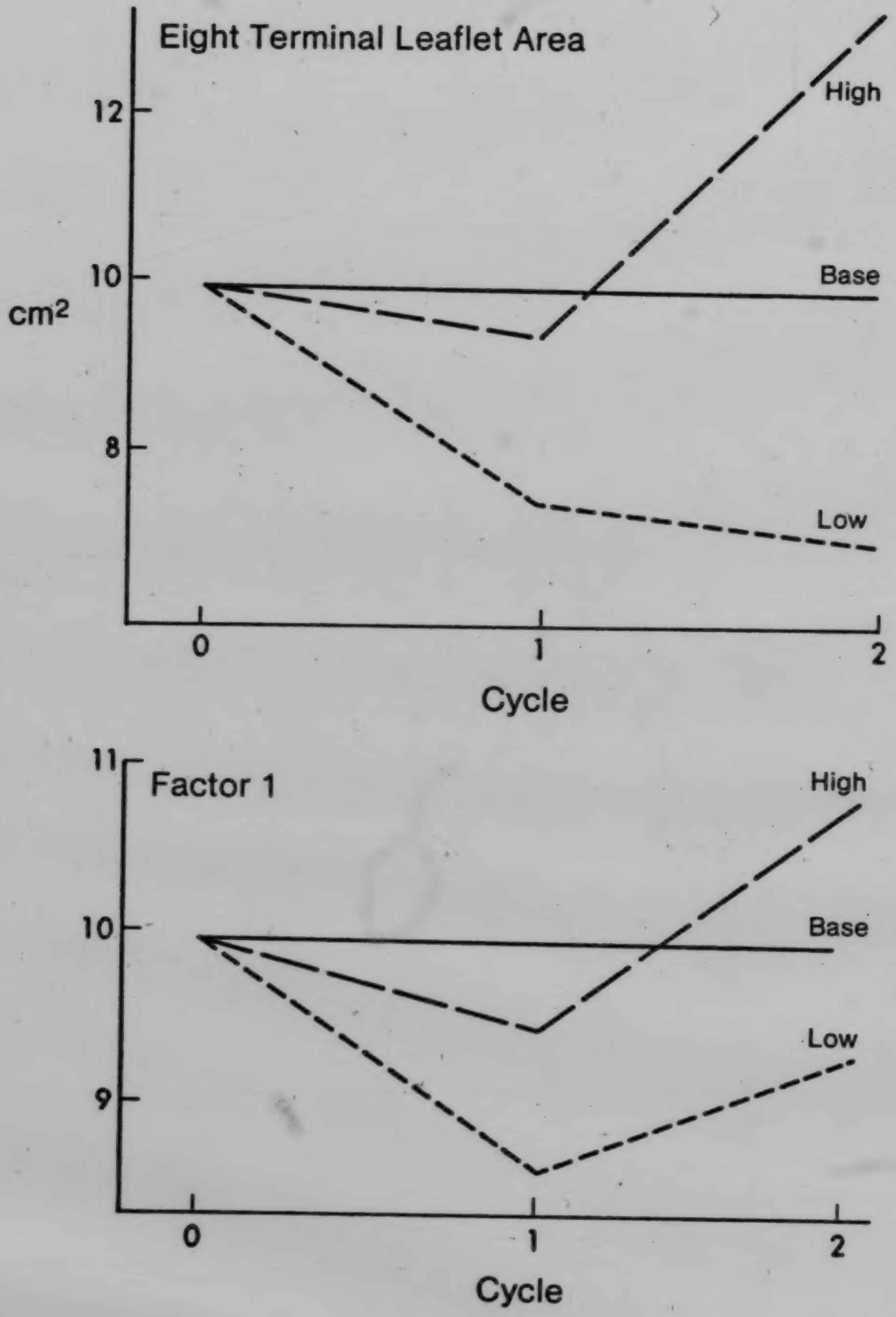


Figure 5. Selection response of the high and low lines for the selection variable and the first principal factor.

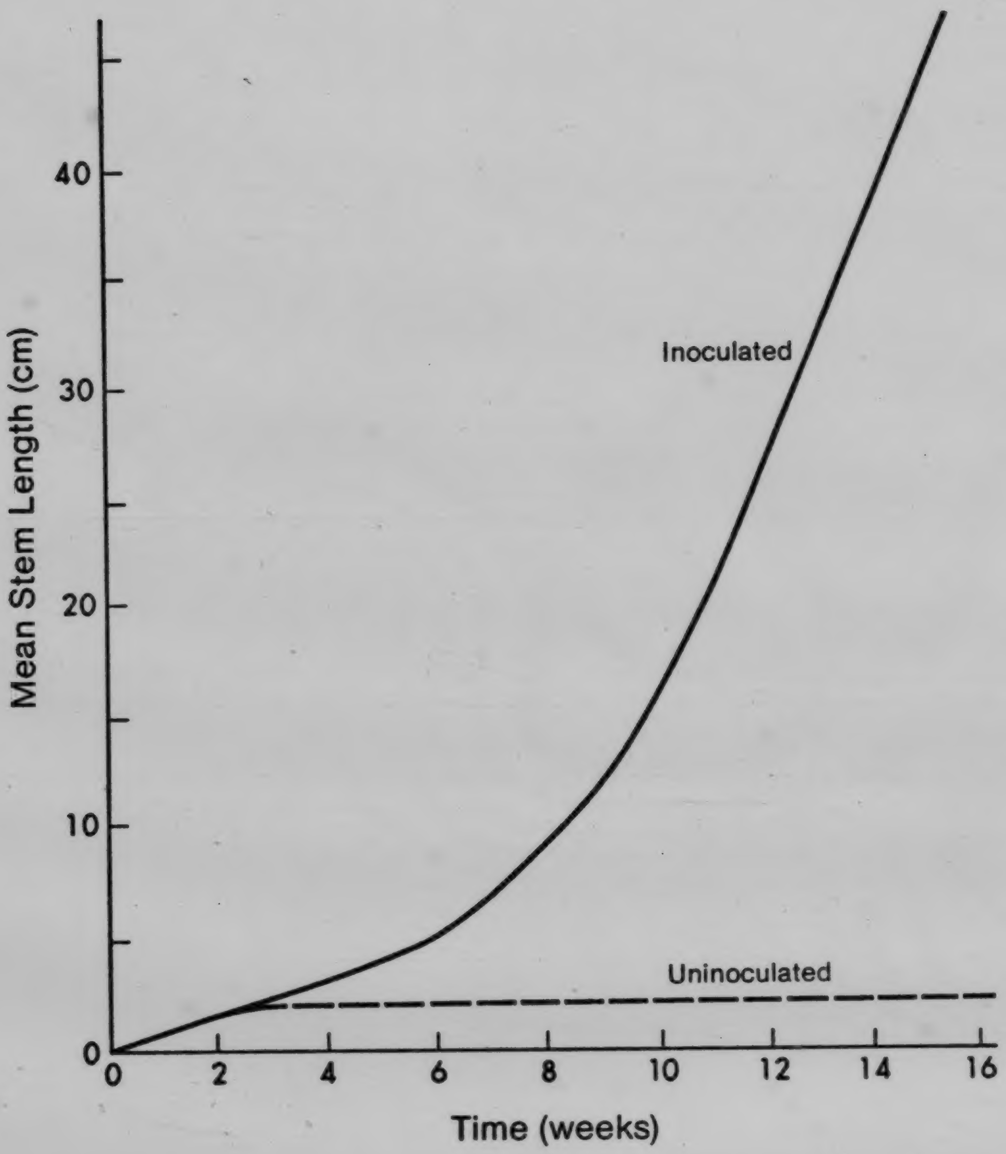


Figure 6. Mean growth curve for the fifteen genotypes of D. sandwicense inoculated with Rhizobium strain B and the uninoculated controls.

presented in Table 11. The pattern which emerged was the consistency with which general effects and average heterosis values exceeded the mean square for genotypes while the other components were less. These data suggested the presence of heterosis and additive genetic variance.

It was observed in the above study that the parental lines appeared to differ in growth before the initiation of the log growth phase and remained different until the study terminated. To confirm these results the primary shoot length was recorded weekly for the parental plants in block 1 of Experiment I. The growth curves for the five inbred lines are presented in Figure 7. The differences between these lines became evident as early as the sixth week after planting.

Hybrid vigor for forage yield has been generally considered to be absent in *Desmodium* when adequately supplied with nitrogen(McWhirter, 1969; Rotar and Chow, 1971). The heterosis present in the estimates of symbiotic nitrogen fixation must be due solely to the processes of nitrogen fixation because the non-nodulated controls did not accumulate dry matter. Therefore, the bottle jar is an effective tool for creating a nitrogen limiting environment for expressing differences in nitrogen fixing potential among population genotypes.

3. Linearity of acetylene reduction.

Acetylene reduction samples were prepared to determine

Table 11. Mean squares expressed as a percent of the genotype mean square of the diallel table genetic components for the initial study.

Component	Primary shoot length 40 day	 60 day	 80 day	 100 day	Leaf areas 2nd node	 5th node	 8th node	 10th node	Total dry weight*
Genotypes	100	100	100	100	100	100	100	100	100
General	125	180	206	160	164	107	109	92	141
Heterosis	90	68	58	76	74	97	96	103	84
Average	242	295	152	270	296	493	131	208	175
Line	54	54	73	99	5	10	96	63	73
Specific	88	34	26	19	86	88	89	114	74

*at 100 days.

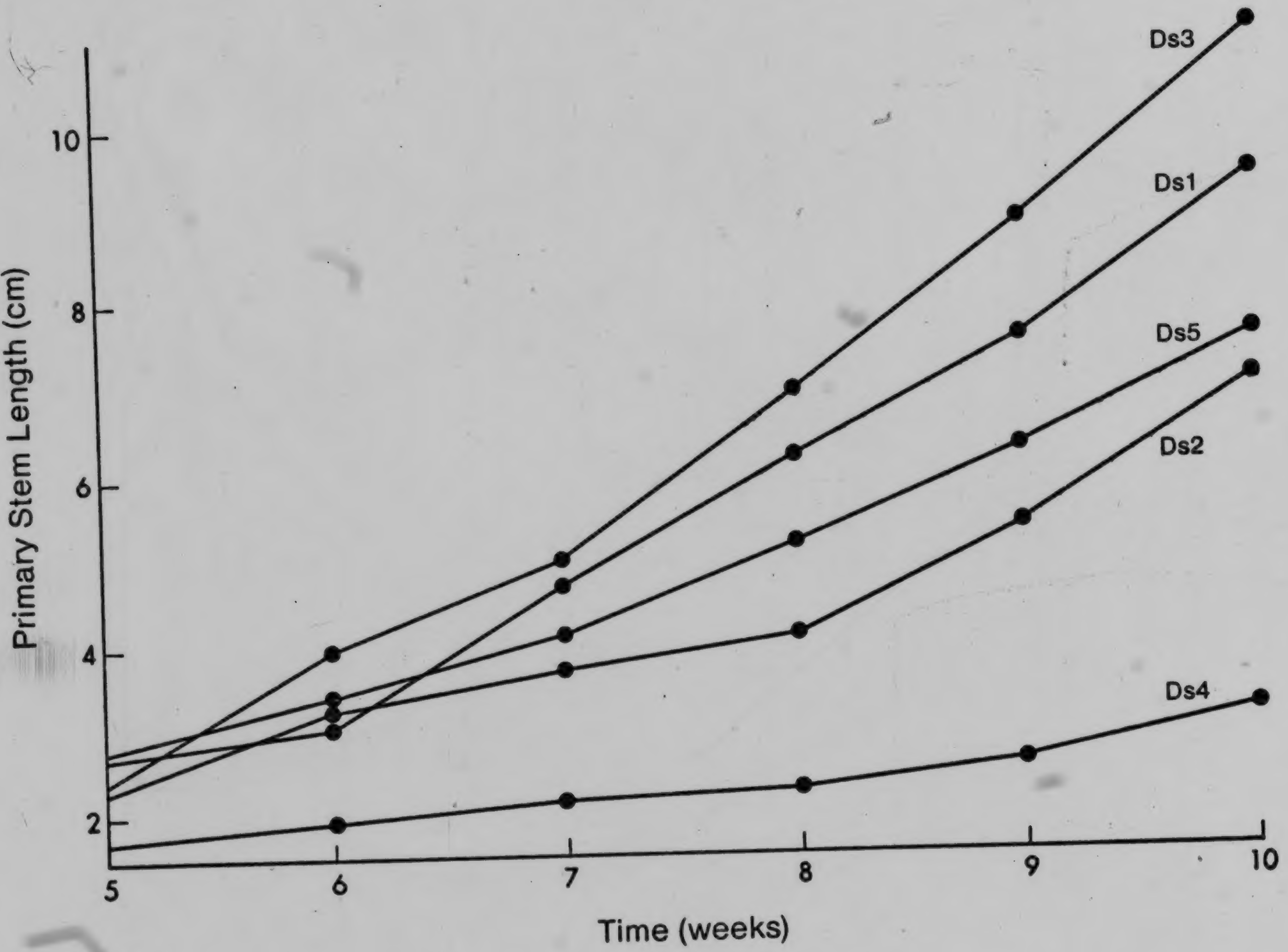


Figure 7. Growth curves for the *D. sandwicense* diallel lines inoculated with *Rhizobium* strain B.

if the reduction of acetylene by Desmodium was linear. The acetylene reduction diagrams for four lines are presented in Figure 8. All samples indicate linear reduction and a sample taken at one hour should adequately represent the amount of acetylene reduced in a one hour period. It should be cautioned that Figure 8 is intended to refer to the linear rate of acetylene reduction only and any comparison among genotypes should be avoided. The acetylene reduction curves for the five diallel lines are presented in Figure 9. These lines also present a linear reduction rate.

The above results suggested that symbiotic nitrogen fixation resulted from nodulated roots; however, the studies did not indicate whether the nodule was the site of nitrogen fixation. Nodules were removed from plant roots and placed separately in one vial and the roots in another. The pairs of vials were prepared and analysed by the acetylene reduction assay. Only the nodule sample indicated any acetylene reduction. It may be concluded that the nodules are the site for nitrogen fixation in Desmodium.

4. Bottle jar design.

During Experiment II in the quantitative genetic variation section (Level 3, Table 1), two short-comings of the bottle jar design became apparent. Firstly, the plant roots would grow into the wick and it was dificult to separate the nodulated roots intact. It was also observed that the environmental variation in the quantitative genetic

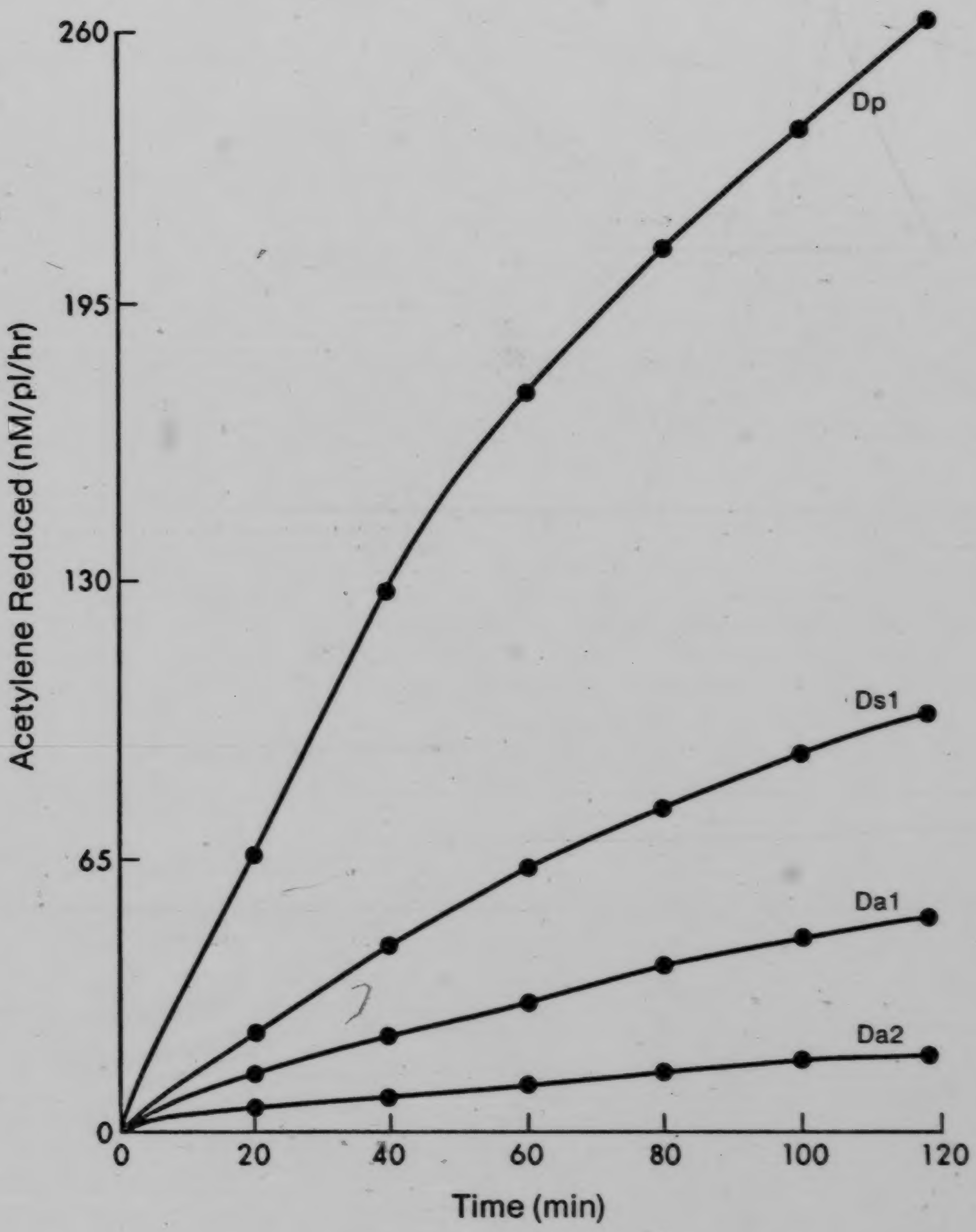


Figure 8. Acetylene reduction curves for several conspicuously different Desmodium species inoculated with Rhizobium strain A.

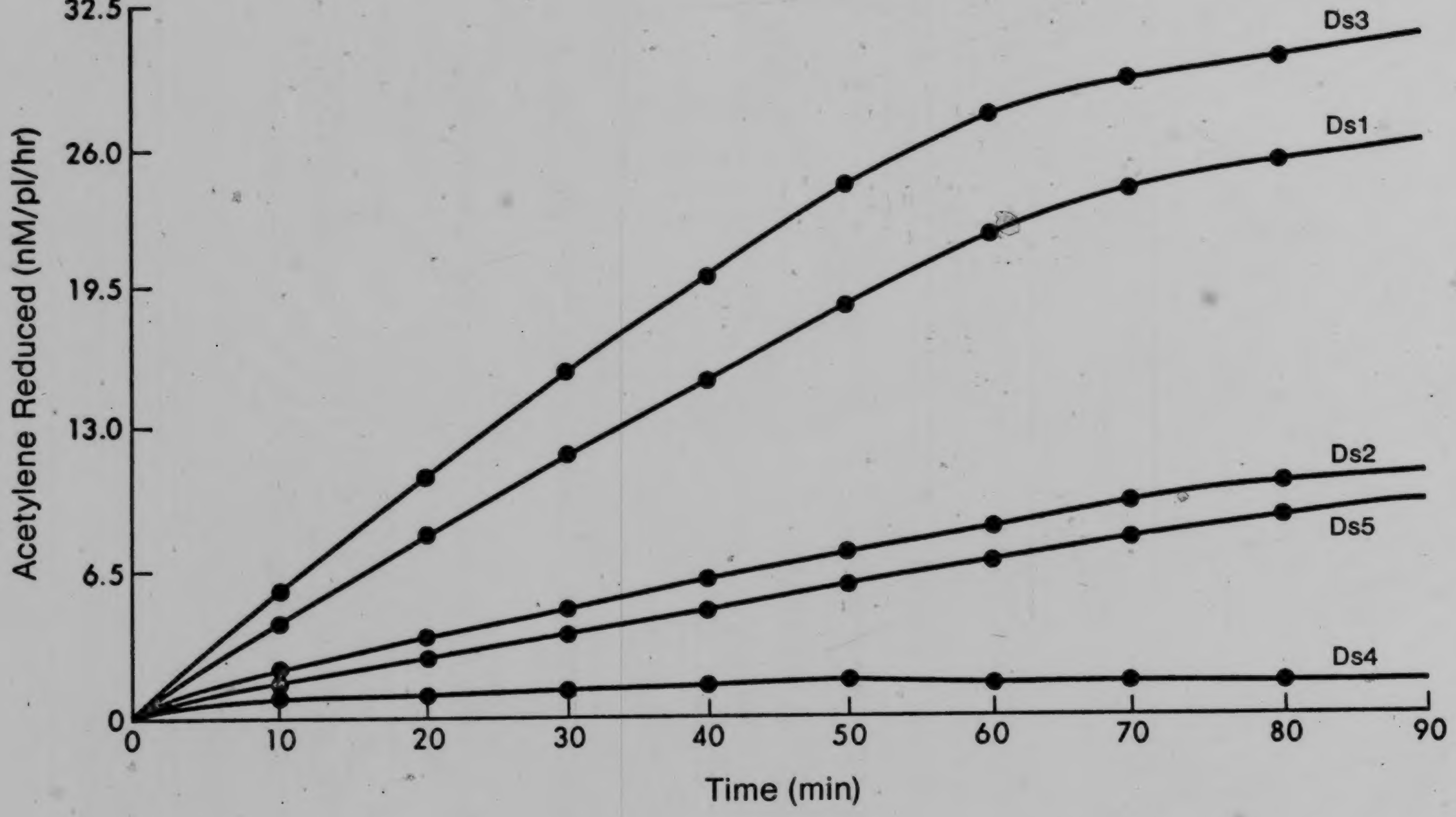


Figure 9. Acetylne reduction curves for the five D. sandwicense diallel lines inoculated with Rhizobium strain B.

studies was excessively large and appeared to be confined largely to within blocks which suggested that the bottle jar design might be manipulated in an effort to reduce this variation. It was suspected that the sand particle size and the distribution of the particles within the bottle jar may contribute to this environmental variation. Within bottle jar variation might be reduced if the sand particle size was more constant.

The analysis of variance for sand sizes is presented in Table 12. Significant differences were recorded for sand size means. The cell means and within-cell differences indicated that some means and differences had larger variances than others (Table 13). The < 4.0 mm sand size means were found to be less for dry weight and shoot length while the < 1.0 mm size had the lowest difference and variance for the within-cell difference between the two plants. Sand sizes with large inter-particle spaces appeared to have larger within cell differences suggesting that perhaps small sand grains assist capillary movement of the nutrient solution.

The germination of the plants in the above experiment was of considerable interest. It was noted that the < 1.0 mm size was the quickest to germinate while the 1.0 - 3.0 mm size emerged 2-3 days later. The other two sizes were intermediate with the < 4.0 mm size being slightly earlier. The sand surface of the 1.0 - 3.0 mm and 1.0 -1.5 mm sizes dried before the rock mulch could be applied while the plant

Table 12. Mean squares from the analysis of variance of the sand size experiment.

Source	df	Dry weight (mg/pl)	Shoot length (cm/pl)
Blocks	5	455.80	2.57
Sand size	3	1718.92*	35.15**
Interaction	15	1191.83	16.99*
Error	23	568.70	5.62

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 13. Means and standard deviations and within-cell differences and standard deviations for the sand size experiment.

Sand Size (mm)	Dry wt. (mg/pl)		Shoot length (cm/pl)	
	Mean	Wcd+	Mean	Wcd+
< 4.0	42.4±11.3	15.2± 9.4	11.9±1.3	1.5±1.1
1.0-3.0	63.4±41.7	37.2±43.3	15.0±4.4	3.6±3.9
1.0-1.5	67.7±26.5	26.0± 9.5	15.5±3.0	2.8±1.1
< 1.0	62.4±13.7	7.2± 4.9	14.2±1.9	0.9±0.5

+within-cell difference.

roots of the 1.0 - 3.0 mm size were found to be predominantly confined to the wick area. This information suggested that seeds planted in the finer sand sizes imbibed water more readily. These results may be due to a uniform capillary movement of the nutrient solution within the < 1.0 mm sand size.

The reduction of the within bottle jar variation would reduce environmental variation; therefore the modified bottle jar was accepted as a less variable container.

5. Analysis of variance of population means for individual epistatic effects.

Voigt *et al.* (1966) stated that an analysis of population means from a cross between two inbred lines "explains as much of the variance among means as possible in terms of additive gene action". Meredith and Laster (1975) report that "the estimates of A and D are independent and uncorrelated only if E is not significant". To investigate these seemingly contradictory statements a coded matrix for the genetic parameters (Mather and Jinks, 1971) was constructed and three types of least squares analysis of this matrix were investigated. The three types of analyses, differing in order of parameter input, were (a) the foward solution least squares analysis which assumed a simple model and which explained the maximum amount of the epistatic effects in terms of additive gene action, (b) a restricted stepwise least squares analysis which assumed a simple model by forcing into the analysis the additive and dominance effect vectors and then explained the remaining variance among the population means by entering the coded interaction vector with the largest partial correlation. The remaining vectors were entered by the same criterion until the variance of the population means was explained. And (c) a free stepwise least squares analysis where the coded parameter vectors were entered in terms of the largest partial correlation. This analysis assumed no model.

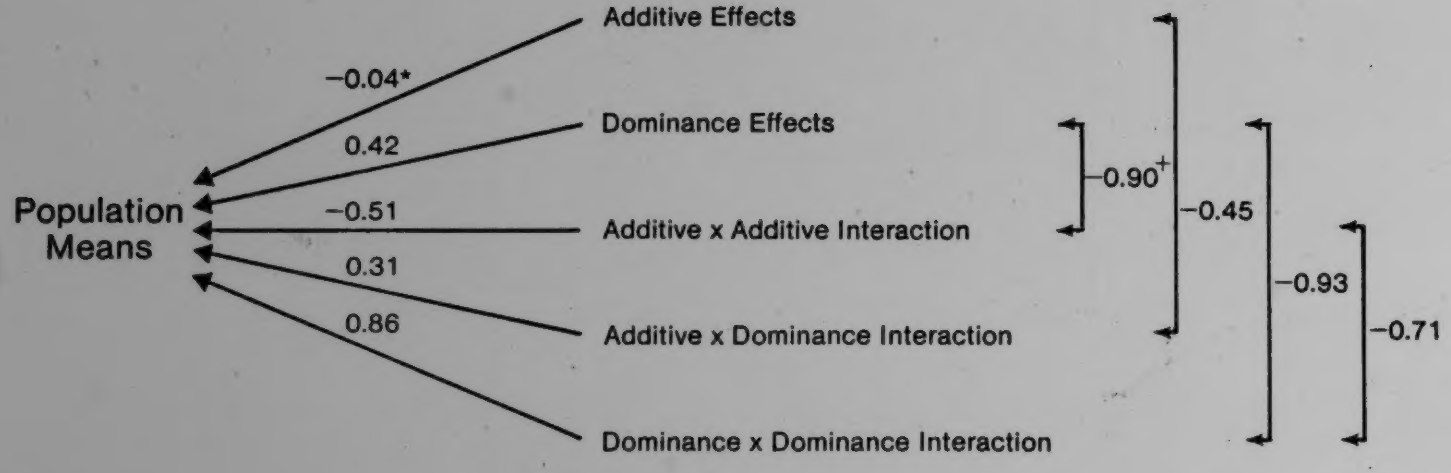
The three analyses of variance of the population means are presented in Table 14. For this study the restricted stepwise least squares analysis has been assumed to be the superior method due to the free partition of the epistatic effects. If the epistatic effects were not partitioned, the restricted stepwise least squares analysis was identical to the forward solution least squares analysis for order of parameter input.

According to Meredith and Laster (1975) the significance of the epistatic effects caused the loss of independence between the additive and dominance effects. To present the relationships between the genetic parameters and the population means, path diagrams (Li, 1975) were constructed for the above analysis. The path diagrams are presented in Figure 10. The additive and dominance effects remain orthogonal for both the direct and the indirect pathways. The non-orthogonality was between the main effects and the interaction effects. Because of the large

Table 14. Mean squares from the genetic analysis of variance of population means for the acetylene reduced (nM/pl/hr) for Experiment IV.

Source	df	Forward solution least squares	Stepwise least squares	
			Restricted	Free
Blocks	7	556.4	556.4	556.4
Populations	5	3689.3**	3689.3**	3689.3**
Additive	1	1923.2* (1+)	1923.2* (1)	7.2 (5)
Dominance	1	8494.4**(2)	8494.4**(2)	8494.4**(1)
Epistatis	3	2676.1	2676.1	3314.9**
Add - Add	1	4995.6**(3)	185.5 (5)	185.5 (4)
Add - Dom	1	359.1 (4)	359.1 (4)	2275.2**(3)
Dom - Dom	1	2676.1**(5)	7484.6**(3)	7484.6**(2)
Error	35	401.8	401.8	401.8

*significant at the 5% level of probability.
**significant at the 1% level of probability.
+order of entry into the regression.


Population Means
Additive Effects
Dominance Effects
Additive x Additive Interaction
Additive x Dominance Interaction
Dominance x Dominance Interaction
−0.04*
0.42
−0.51
0.31
0.86
−0.90+
−0.45
−0.93
−0.71

*Standardized Regression Coefficient
+Correlation Coefficient

Figure 10. Path diagrams of the analysis of population means for acetylene reduced (nM/pl/hr) for Experiment IV).

coefficients of determination (r^2) between the main effects and the interaction effects the analysis of variance of population means should be interpreted with caution, since a large proportion of the main effects may be explained as interaction effects. However, the analysis is of some value because the main effects were orthogonal even in the presence of significant epistatic effects and also provided an estimate of the significance of the interaction parameters.

6. Strain-specific ineffective nodulation interaction.

Several lines of conspicuously different Desmodium species were tested with the selected strains to determine the type of host-strain interaction. The interactions are presented in Table 15. D. aparines lines Da1, Da2 and Da3 were found to be ineffectively nodulated by strain B. These lines also exhibited small seed size and all originated from Costa Rica or Guatemala (Imrie, 1973). The D. aparines lines Da4 and Da5 exhibited effective nodulation interactions with strain B as well as large seeds. These lines were originally collected from Colombia and Mexico, respectively.

B. Qualitative genetic variation.

The area of study of the inheritance of nitrogen fixation which has commanded the greatest interest has been the study of non-nodulating or strain-specific ineffective nodulation. These conditions were found to be produced by

Table 15. Strain-specific interactions of *Desmodium* lines with *Rhizobium* strains.

	Rhizobium Strain	
Lines	A	B
Ds1	E	E
Ds2	E	E
Ds3	E	E
Ds4	E	E
Ds5	E	E
Da1	E	I
Da2	E	I
Da3	E	I
Da4	E	E
Da5	E	E
Dp	E	E
Du	E	E
Dsp	E	E*
Dse	I?	E*

E - effective nodulation (green plant).
I - ineffective nodulation (yellow-chlorotic plant).
? - not verified.
* - appears to be pH sensitive.

major genes. In preliminary experiments of this study in Desmodium it was noted that a strain-specific ineffective nodulation interaction was found between some *D. aparines* lines when inoculated with strain B. The numbers of observations for each of the scores of the F_2 plants for nodulation interaction and spotted leaf character are presented in Table 16. The statistical analyses of the F_2 plants suggested that inheritance of this strain-specific ineffective nodulation interaction was conditioned by a dominant allele at a single locus.

The analysis of the leaf flecking character indicated the presence of complementary gene interaction in the Ds1 X Da2 cross. The Ds1 X Da1 cross appeared to be segregating at a single locus as the data conformed to a 3:1 segregation ratio. The joint segregation of the ineffective/effective nodulation and spotted/plain leaf character for the Ds1 X Da cross suggested that the two characters were independent in inheritance.

To test the hypothesis that a single dominant gene conditioned the strain-specific ineffective nodulation interaction between some *D. aparines* lines and strain B, the backcrosses to Ds1 were grown and the plants scored for green vs yellow-chlorotic color and for spotted vs plain leaves. The character scores for the backcross plants are presented in Table 17. These data for nodulation conformed to the expectations of a single gene hypothesis thereby suggesting the single gene hypothesis for the strain-

Table 16. Observed nodulation and leaf fleck scores and Chi-square probabilities for the F_2 plants of Desmodium crosses inoculated with strain B.

	Nodulation			Leaf fleck			Joint segregation				
Cross	Ineff. (I)	Eff. (E)	Chi-sq. prob. (3:1)	Spotted (S)	Plain (P)	Chi-sq. prob. (3:1)	I/S	I/P	E/S	E/P	Chi-sq. probability 9:3:3:1
Ds1 X Da1	88	36	.80-.70	33	91	.80-.70	61	27	30	6	.20-.10
Ds1 X Da2	91	24	.80-.70	68	47	.70-.60*	38	51	7	17	-------

--calculation not available.
*complementary gene interaction (9:7).

Table 17. Observed nodulation and leaf fleck scores and Chi-square probabilities for the backcross plants of the *Desmodium* crosses inoculated with strain B.

	Nodulation			Leaf fleck			Joint segregation				
Cross	Ineff. (I)	Eff. (E)	Chi-sq. prob. (1:1)	Spotted (S)	Plain (P)	Chi-sq. Prob. (1:1)	I/S	I/P	E/S	E/P	Chi-sq. probability 1:1:1:1
Ds1 X Da1	35	36	.98-.95	42	29	.30-.20	21	14	21	15	.50-.40
Ds1 X Da2	41	32	.50-.40	32	41	.50-.40*	15	26	17	15	.30-.20
Dev. Chi-sq.	76	68	.70-.60	74	70	.90-.80	36	40	38	30	.70-.60
Heterogeneity Chi-sq.			.60-.50			.20-.10					.20-.10

*actual segregation ratio (1:1:1:1).

specific ineffective nodulation in *Desmodium*. These results have been published by Pinchbeck (1978) (Appendix D) and are in accord with the published results for soybean where three single dominant genes were reported for strain-specific ineffective interactions (Caldwell, 1966; Vest, 1970; Vest and Caldwell, 1972).

Seventy randomly selected ineffectively nodulated F_2 plants were inspected for nodulation characteristics and replanted in pots and supplied with nitrogen. All ineffectively nodulated plants responded to nitrogen fertilizer. The roots of the ineffectively nodulated plants exhibited various degrees of nodule formation. The majority of nodules were small whereas a few were near normal size. All nodules lacked pink pigmentation, a sign of an active nodule (Vincent, 1970). These nodules appeared to be similar to those reported by Caldwell (1966) and Vest (1970) for the Rj_2 and Rj_3 genes in soybean.

The symbols N_1/n_1 are suggested for the designation of the *D. aparines* strain-specific ineffective gene. *D. sandwicense* has the genotype n_1, n_1 while the ineffectively nodulated *D. aparines* lines have the genotype N_1, N_1.

Similar phenotypes for ineffective nodulation interaction were exhibited by Da1 and Da2 lines . However, it is not known whether these phenotypes were conditioned by similar or different genes. To confirm this question a standard test of functional allelism (Srb, Owen and Edgar, 1965) would need to be applied to the F_2 generation of a Da1

X Da2 cross.

Experimentation for the relationship between the N_1 gene and seed size was not pursued in this study as a single gene influencing seed size has not been reported. This avenue would be best examined by exploring the fertile D. aparines X D. uncinatum cross since both species exhibited the extremes for seed size and have different genotypes for the N_1 gene.

C. Quantitative genetic variation.

A great need in international agriculture has been to increase total symbiotic nitrogen fixation. The traditional method of increasing total fixation has been manipulation of the Rhizobium strains used for inoculum (Vincent, 1974). An alternate research avenue is to establish and exploit the variability within the host. It was an objective of this study to investigate the hypothesis that there is variation within Desmodium for total symbiotic nitrogen fixation and that this variation has a heritable basis. The diallel cross and a cross between two inbred lines have been employed as quantitative genetic designs to establish this genetic variation.

1. Diallel cross.

a. Experiment I.

The analysis of variance* for two characters from

Experiment I is presented in Table 18. Tukey's test for non-additivity of the data (Snedecor and Cochran, 1967) indicated that a transformation of the data was necessary. Vincent (1970) suggested that dry weight accumulation data should be analysed in the logrithmetic scale. Non-significance for genotypes was recorded for dry weight and the cause appeared to be block 2 which was significantly different from the remainder (Table 19). This block may have differed for three reasons: (a) due to the mechanical failure of the gas chromatograph this block was harvested one week later than the remainder, (b) block 2 may have been inoculated with a contaminated broth of Rhizobium since the values of parent 4 and some of its crosses are greatly increased, or (c) that the bottle type used in block 2 created a different environment thereby affecting the dry weight accumulation results. Block 2 was deleted from further analyses.

Principal components analysis of the collected characters indicated that the characters were highly correlated and related to one principal factor (Table 20).

The genotype means for the characters measured (transformed to $\log_{10}$) are presented in Table 21. The diallel genotypes revealed that most crosses exhibited heterosis suggesting that heterosis may be a characteristic

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*Due to a mechanical failure of the gas chromatograph, the acetylene reduction data were not collected for blocks 2 and 3 of this study. Therefore acetylene reduction character was not reported for Experiment I.

Table 18. Mean squares of two original characters for Experiment I.

Source	d.f.	Total shoot length (cm/pl)	Total dry weight (mg/pl)
Blocks	3	2084**	48750**
Genotypes	14	109*	2542
Error	42	44	1873
Non-additivity	1	610**	23439**
Residual	41	31	1347

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 19. Dry weight (mg/pl) of the diallel genotypes for Experiment I.

Genotype	Block 1	2	3	4
Ds1	70	136	51	111
Ds2	41	63	31	35
Ds3	91	118	43	39
Ds4	11	281	51	37
Ds5	45	142	49	65
Ds1 x Ds2	64	109	107	111
Ds1 x Ds3	115	163	74	102
Ds1 x Ds4	48	316	117	120
Ds1 x Ds5	60	137	81	112
Ds2 x Ds3	73	194	123	54
Ds2 x Ds4	42	157	34	112
Ds2 x Ds5	33	214	66	122
Ds3 x Ds4	52	300	65	96
Ds3 x Ds5	55	220	48	141
Ds4 x Ds5	40	170	51	136
Block means	56	181	66	93

Table 20. Correlations and principal eigenvector loadings for Experiment I.

Character		Correlations						Eigenvector
		1	2	3	4	5	6	
Main shoot length	1	1.00						.86
Secondary shoot length	2	.43	1.00					.77
Total shoot length	3	.91	.71	1.00				.97
Leaf dry weight	4	.73	.75	.87	1.00			.96
Shoot dry weight	5	.89	.68	.96	.94	1.00		.99
Total dry weight	6	.78	.74	.90	.99	.97	1.00	.98

Table 21. Log_{10} means and standard error of the difference between two means for Experiment I.

Genotype	Main shoot length (cm/pl)	Secondary shoot length (cm/pl)	Total shoot length (cm/pl)	Leaf dry weight (mg/pl)
Ds1	1.090	.768	1.740	1.271
Ds2	0.907	.417	1.408	0.962
Ds3	1.052	.258	1.601	1.125
Ds4	0.826	.067	1.331	0.778
Ds5	1.024	.371	1.591	1.123
Ds1 x Ds2	1.146	.939	1.825	1.379
Ds1 x Ds3	1.128	.820	1.849	1.385
Ds1 x Ds4	1.010	.735	1.813	1.349
Ds1 x Ds5	1.083	.759	1.795	1.249
Ds2 x Ds3	1.102	.736	1.756	1.319
Ds2 x Ds4	0.985	.645	1.638	1.124
Ds2 x Ds5	1.056	.723	1.685	1.198
Ds3 x Ds4	1.097	.364	1.711	1.234
Ds3 x Ds5	1.102	.565	1.724	1.276
Ds4 x Ds5	1.055	.576	1.701	1.184
se of d.	0.084	0.385	0.141	0.161

Table 21 (cont). Log_{10} means and standard error of the difference between two means for Experiment I.

Genotype	Shoot dry weight (mg/pl)	Total dry weight (mg/pl)	Nitrogen fixation "factor"
Ds1	1.261	1.866	0.366
Ds2	1.011	1.549	-0.083
Ds3	1.089	1.728	-0.404
Ds4	0.840	1.439	-1.706
Ds5	1.078	1.719	-0.362
Ds1 x Ds2	1.346	1.960	0.784
Ds1 x Ds3	1.290	1.980	0.725
Ds1 X Ds4	1.236	1.943	0.542
Ds1 x Ds5	1.246	1.912	0.419
Ds2 X Ds3	1.249	1.895	0.438
Ds2 x Ds4	1.120	1.735	-0.206
Ds2 x Ds5	1.206	1.808	0.121
Ds3 x Ds4	1.139	1.837	0.041
Ds3 x Ds5	1.197	1.856	0.222
Ds4 x Ds5	1.165	1.814	0.004
se of d.	0.105	0.148	0.581

in *Desmodium* for total symbiotic nitrogen fixed.

The diallel analyses of variance for the $\log_{10}$ transformed characters in Experiment I are presented in Table 22. All characters including the "Nitrogen fixation" factor were noted to have a similar analysis pattern. The significant mean squares for genotypes suggested that genetic variation for the characters existed within the *D. sandwicense* population. Significant general effects indicated that a portion of this variation was heritable. Although heterosis was not significant, average heterosis was shown to be highly significant indicating that the F_1 progeny means were significantly different from the parental means.

The estimates of the G.C.A. effects and variety constants are presented in Table 23. Line Ds1 exhibited positive G.C.A. effects and variety constants for total dry weight while line Ds4 was found to have a negative G.C.A. effect and variety constant.

Estimates of the heterosis constants revealed that, in general, the line and specific heterosis constants were small in magnitude in comparison to the average heterosis constant (Table 24). These results were expected since line and specific heterosis effects were not significant in the diallel analysis of variance.

The estimates of the S.C.A. effects failed to define any hybrid which was superior to the remainder (Table 25). The negative S.C.A. estimates for inbred lines suggested

Table 22. Mean squares from the diallel analysis of variance for Experiment I.

Source	df	Main shoot length (cm/pl)	Secondary shoot length (cm/pl)	Total shoot length (cm/pl)	Leaf dry weight (mg/pl)	Shoot dry weight (mg/pl)	Total dry weight (mg/pl)	Nitrogen fixation "factor"
Blocks	2	.437**	.483**	.143*	.455**	.455**	.194*	6.075**
Genotypes	14	.023*	.610**	.065*	.079	.048*	.068*	1.332*
General	4	.041*	1.105**	.099*	.128*	.091**	.110*	2.201**
Heterosis	10	.015	.413	.051	.059	.031	.052	.985
Average	1	.114**	3.593**	.465**	.473**	.268**	.457**	8.594**
Line	4	.005	.108	.011	.020	.006	.010	.231
Specific	5	.004	.020	.002	.008	.003	.004	.066
Error	28	.011	.222	.030	.039	.016	.033	.507
Non-additivity	1	.001	1.508**	.000	.003	.004	.001	.047
Residual	27	.010	.175	.031	.040	.017	.034	.524

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 23. Estimates of G.C.A. effects and variety constants for Experiment I.

	Main shoot length (cm/pl)	Secondary shoot length (cm/pl)	Total shoot length (cm/pl)	Leaf dry weight (mg/pl)	Shoot dry weight (mg/pl)	Total dry weight (mg/pl)	Nitrogen fixation "factor"
Griffing's G.C.A. effects							
g(Ds1)	.049	.316	.093	.099	.103	.101	.457
g(Ds2)	-.029	.084	-.007	-.049	-.034	-.046	-.119
g(Ds3)	.033	-.104	.009	.025	.040	.030	.088
g(Ds4)	-.058	-.305	-.093	-.078	-.105	-.087	-.433
g(Ds5)	-.006	.009	-.003	.003	-.004	.002	.006
se[g(i)]	.020	.092	.025	.034	.039	.035	.139
se[g(i)-g(j)]	.030	.146	.039	.054	.061	.056	.220
Gardner's variety constants							
mean	.980	-.064	1.534	1.052	1.056	1.660	-0.618
v(Ds1)	.111	.720	.206	.219	.205	.206	-.618
v(Ds2)	-.073	.046	-.126	-.090	-.044	-.111	-.365
v(Ds3)	.072	-.201	.067	.073	.033	.068	.214
v(Ds4)	-.154	-.735	-.203	-.274	-.216	-.221	-1.089
v(Ds5)	.044	.170	.057	.071	.023	.059	.256

Table 24. Estimates of Gardner's average, line and specific heterosis constants for Experiment I.

Heterosis Constant	Main shoot length (cm/pl)	Secondary shoot length (cm/pl)	Total shoot length (cm/pl)	Leaf dry weight (mg/pl)	Shoot dry weight (mg/pl)	Total dry weight (mg/pl)	Nitrogen fixation "factor"
h(ave)	.107	.310	.215	.218	.164	.214	.927
h(Ds1)	-.015	-.027	-.009	-.015	-.022	-.004	-.081
h(Ds2)	.018	.079	.031	.025	.037	.023	.150
h(Ds3)	-.008	-.028	-.020	.008	-.018	-.010	-.044
h(Ds4)	.044	.013	.056	.074	.035	.055	.260
h(Ds5)	-.039	-.038	-.060	-.093	-.032	-.064	-.285
h(Ds1,Ds2)	.038	-.016	.012	.034	.031	.020	.097
h(Ds1,Ds3)	-.027	.051	-.009	-.024	-.008	-.018	-.059
h(Ds1,Ds4)	.016	.021	.014	.048	.009	.025	.106
h(Ds1,Ds5)	-.027	-.056	-.018	-.058	-.032	-.027	-.145
h(Ds2,Ds3)	.007	.037	.024	.024	.016	.030	.098
h(Ds2,Ds4)	-.050	.001	-.035	-.064	-.041	-.051	-.197
h(Ds2,Ds5)	.005	-.022	-.002	.006	-.007	.002	.002
h(Ds3,Ds4)	.016	-.094	-.007	-.018	-.007	-.005	-.046
h(Ds3,Ds5)	.004	.006	-.009	.018	.000	-.006	.006
h(Ds4,Ds5)	.018	.072	.028	.034	.039	.031	.137

Table 25. Estimates of Griffing's S.C.A. effects for Experiment I.

S.C.A. effect	Main shoot length (cm/pl)	Secondary shoot length (cm/pl)	Total shoot length (cm/pl)	Leaf dry weight (mg/pl)
s(Ds1)	-.059	-.311	-.090	-.136
s(Ds2)	-.086	-.521	-.140	-.171
s(Ds3)	-.064	-.393	-.094	-.127
s(Ds4)	-.109	-.525	-.139	-.139
s(Ds5)	-.038	-.247	-.081	-.092
se[s(i)]	.041	.188	.051	.069
se[s(i)-s(j)]	.055	.252	.068	.093
s(Ds1,Ds2)	.076	.146	.095	.097
s(Ds1,Ds3)	-.004	.195	.023	.047
s(Ds1,Ds4)	.068	.283	.071	.113
s(Ds1,Ds5)	-.022	-.002	-.009	.015
s(Ds2,Ds3)	.048	.331	.081	.103
s(Ds2,Ds4)	.021	.390	.055	.087
s(Ds2,Ds5)	.028	.176	.050	.054
s(Ds3,Ds4)	.072	.158	.058	.086
s(Ds3,Ds5)	.013	.102	.026	.018
s(Ds4,Ds5)	.057	.218	.095	.097
se[s(i,j)]	.052	.238	.064	.088
se[s(i,j)-s(i,k)]	.078	.356	.096	.132
se[s(i,j)-s(k,l)]	.071	.325	.088	.120

Table 25 (cont). Estimates of Griffing's S.C.A. effects for Experiment I.

S.C.A. effect	Shoot dry weight (mg/pl)	Total dry weight (mg/pl)	Nitrogen fixation "factor"
s(Ds1)	-.132	-.140	-.549
s(Ds2)	-.167	-.162	-.747
s(Ds3)	-.152	-.134	-.580
s(Ds4)	-.192	-.190	-.840
s(Ds5)	-.065	-.088	-.374
se[s(i)]	.079	.072	.284
se[s(i)-s(j)]	.106	.097	.381
s(Ds1,Ds2)	.113	.102	.446
s(Ds1,Ds3)	.044	.046	.179
s(Ds1,Ds4)	.154	.126	.518
s(Ds1,Ds5)	-.047	.006	-.044
s(Ds2,Ds3)	.115	.108	.468
s(Ds2,Ds4)	.066	.065	.346
s(Ds2,Ds5)	.039	.049	.234
s(Ds3,Ds4)	.102	.092	.386
s(Ds3,Ds5)	.043	.023	.127
s(Ds4,Ds5)	.096	.097	.431
se[s(i,j)]	.100	.091	.359
se[s(i,j)-s(i,k)]	.149	.137	.538
se[s(i,j)-s(k,l)]	.136	.125	.491

that homozygous lines may be exhibiting inbreeding depression for total symbiotic nitrogen fixed.

b. Experiment II.

The data from Experiment I indicated that total symbiotic nitrogen fixed appeared to be heritable. To further investigate the hypothesis that there exists genetic variation for nitrogen fixation, the diallel cross was grown with *Rhizobium* strain A to determine if the mode of inheritance was consistent for a different strain.

Principal components analysis of the characters measured for Experiment II revealed that all characters were highly correlated and related to one factor (Table 26).

The genotype means from the $\log_{10}$ transformed characters are presented in Table 27. An examination of the individual genotype means revealed that every cross exhibited heterobeltiosis except cross Ds2 x Ds3 which portrayed only complete heterosis for the acetylene reduction assay.

The diallel analyses of variance for the $\log_{10}$ transformed characters for Experiment II are given in Table 28. There were significant differences among genotypes which confirmed that genetic variation was present in the diallel population. General effects, heterosis and average heterosis components were significant while line and specific heterosis were non-significant. The genetic variance was confined to among parental lines and between the progeny and

Table 26. Correlations coefficients and principal eigenvector loadings for Experiment II.

Character		Correlations				Eigenvector
		1	2	3	4	
Leaf dry weight	1	1.00				.99
Shoot dry weight	2	.97	1.00			.99
Total dry weight	3	.99	.98	1.00		.99
Acetylene reduced	4	.91	.90	.92	1.00	.93

Table 27. Log_{10} means and standard error of the difference between two means for Experiment II.

Genotype	Leaf dry weight (mg/pl)	Shoot dry weight (mg/pl)	Total dry weight (mg/pl)
Ds1	1.627	1.056	1.732
Ds2	1.660	1.147	1.777
Ds3	1.838	1.355	1.962
Ds4	1.749	1.127	1.842
Ds5	1.795	1.272	1.909
Ds1 x Ds2	1.842	1.308	1.954
Ds1 x Ds3	1.948	1.452	2.070
Ds1 x Ds4	2.003	1.466	2.115
Ds1 x Ds5	1.950	1.457	2.073
Ds2 x Ds3	1.914	1.425	2.037
Ds2 x Ds4	2.035	1.494	2.145
Ds2 x Ds5	2.036	1.525	2.153
Ds3 x Ds4	2.214	1.683	2.327
Ds3 x Ds5	2.141	1.640	2.261
Ds4 x Ds5	1.941	1.346	2.041
se of d.	0.164	0.199	0.172

Table 27 (cont). Log_{10} means and standard error of the difference between two means for Experiment II.

Genotype	Acetylene reduced (nmoles/pl/hr)	Nitrogen fixation "factor"
Ds1	1.648	1.831
Ds2	1.488	1.831
Ds3	1.864	1.847
Ds4	1.506	1.835
Ds5	1.726	1.842
Ds1 x Ds2	1.759	1.845
Ds1 x Ds3	1.880	1.853
Ds1 x Ds4	1.835	1.855
Ds1 x Ds5	1.996	1.855
Ds2 x Ds3	1.863	1.851
Ds2 x Ds4	1.898	1.857
Ds2 x Ds5	2.122	1.861
Ds3 x Ds4	2.083	1.870
Ds3 x Ds5	2.030	1.866
Ds4 x Ds5	1.732	1.849
se of d.	0.206	0.460

Table 28. Mean squares from the diallel analysis of variance for Experiment II.

Source	df	Leaf dry weight (mg/pl)	Shoot dry weight (mg/pl)	Total dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Nitrogen fixation "factor"
Blocks	3	2.137**	2.401**	2.209**	4.778**	17.427**
Genotypes	14	.218**	.261**	.225*	.290**	1.559**
General	4	.169*	.212*	.172*	.241*	1.150*
Heterosis	10	.237**	.280**	.246**	.310**	1.722**
Average	1	1.923**	2.214**	1.989**	1.999**	13.622**
Line	4	.018	.017	.017	.046	.084
Specific	5	.076	.103	.081	.183	.652
Blocks x genotypes	42	.065	.090	.070	.138*	.530
Blocks x general	12	.061	.062	.060	.118*	.423
Blocks x heterosis	30	.067	.102	.074	.146*	.572
Blocks x average	3	.095	.162	.108	.037	.657
Blocks x line	12	.055	.089	.062	.157	.517
Blocks x specific	15	.072	.100	.077	.159*	.599
Error	56	.054	.079	.059	.085	.423

*significant at the 5% level of probability.
**significant at the 1% level of probability.

parental means. Block x genotype interactions were not significant for dry weight characters but significant for the acetylene reduction assay. This component was distorted because of a problem of the separation of the nodulated roots from the lamp wick (Figure 2) in the preparation of the plants for the assay. As a result of this problem the bottle jar design was modified (Figure 3).

The estimates of the G.C.A. effects and variety constants are presented in Table 29 and line Ds3 was shown to have the greatest G.C.A. effect and variety constant for all characters. Line Ds1, however, exhibited a negative G.C.A. effect and variety constant which is the reverse of Experiment I. These results were of interest since line Ds3 was a Ds1 backcross line from an original Ds1 x D. aparines (Da1) cross. Since strain A was isolated from D. aparines, a strain x genotype interaction may be suggested.

The estimates of the heterosis constants again revealed that average heterosis was much larger than either the estimates of the line or specific heterosis constants (Table 30).

The S.C.A. effect estimates revealed inbreeding depression in the inbred lines for all characters (Table 31). The cross Ds3 x Ds4 showed considerable S.C.A. effect for the dry weight characters. However, the cross Ds2 x Ds5 exhibited a superior S.C.A. effect for the acetylene reduction assay. The cross Ds2 x Ds5 also had a larger acetylene reduction assay S.C.A. value relative to its dry

Table 29. Estimates of G.C.A. effects and variety constants for Experiment II.

	Leaf dry weight (mg/pl)	Shoot dry weight (mg/pl)	Total dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Nitrogen fixation "factor"
Griffing's G.C.A. effects					
g(Ds1)	-.069	-.072	-.069	-.030	-.160
g(Ds2)	-.047	-.036	-.045	-.051	-.117
g(Ds3)	.060	.087	.066	.088	.190
g(Ds4)	.030	-.008	.022	-.059	.005
g(Ds5)	.026	.030	.027	.051	.083
se[g(i)]	.028	.034	.029	.035	.078
se[g(i)-g(j)]	.044	.053	.046	.055	.123
Gardner's variety constants					
mean	1.734	1.191	1.845	1.646	-.476
v(Ds1)	-.106	-.136	-.113	.001	-.244
v(Ds2)	-.074	-.044	-.068	-.158	-.211
v(Ds3)	.104	.164	.118	.218	.373
v(Ds4)	.015	-.065	-.002	-.140	-.098
v(Ds5)	.061	.081	.065	.080	.180

Table 30. Estimates of Gardner's average, line and specific heterosis constants for Experiment II.

Heterosis constant	Leaf dry weight (mg/pl)	Shoot dry weight (mg/pl)	Total dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Nitrogen fixation "factor"
h(ave)	.269	.288	.273	.274	.715
h(Ds1)	-.036	-.011	-.030	-.070	-.088
h(Ds2)	-.024	-.033	-.027	.067	-.028
h(Ds3)	.017	.012	.016	-.050	.007
h(Ds4)	.053	.056	.054	.027	.125
h(Ds5)	-.011	-.024	-.013	.027	-.016
h(Ds1,Ds2)	-.011	-.038	-.017	-.079	-.085
h(Ds1,Ds3)	-.035	-.044	-.037	-.029	-.094
h(Ds1,Ds4)	.028	.042	.031	.029	.085
h(Ds1,Ds5)	.017	.040	.023	.079	.094
h(Ds2,Ds3)	-.097	-.093	-.095	-.103	-.246
h(Ds2,Ds4)	.032	.047	.035	.034	.094
h(Ds2,Ds5)	.075	.084	.077	.148	.237
h(Ds3,Ds4)	.082	.086	.082	.148	.246
h(Ds3,Ds5)	.050	.050	.049	-.016	.093
h(Ds4,Ds5)	-.142	-.174	-.149	-.211	-.425

Table 31. Estimates of Griffing's S.C.A. effects for Experiment II.

S.C.A. effect	Leaf dry weight (mg/pl)	Shoot dry weight (mg/pl)	Total dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Nitrogen fixation "factor"
s(Ds1)	-.149	-.183	-.157	-.122	-.401
s(Ds2)	-.158	-.163	-.159	-.240	-.453
s(Ds3)	-.194	-.202	-.196	-.140	-.483
s(Ds4)	-.225	-.240	-.228	-.205	-.584
s(Ds5)	-.170	-.172	-.171	-.206	-.463
se[s(i)]	.057	.069	.059	.071	.159
se[s(i)-s(j)]	.076	.092	.079	.095	.213
s(Ds1, Ds2)	.045	.033	.042	.011	.087
s(Ds1, Ds3)	.044	.053	.047	-.006	.098
s(Ds1, Ds4)	.128	.163	.136	.095	.344
s(Ds1, Ds5)	.080	.116	.089	.145	.273
s(Ds2, Ds3)	-.011	-.009	-.010	-.002	-.020
s(Ds2, Ds4)	.139	.155	.141	.179	.388
s(Ds2, Ds5)	.145	.148	.145	.293	.450
s(Ds3, Ds4)	.211	.221	.213	.226	.560
s(Ds3, Ds5)	.143	.140	.142	.062	.327
s(Ds4, Ds5)	-.028	-.060	-.034	-.089	-.124
se[s(i, j)]	.072	.087	.075	.090	.201
se[s(i, j)-s(i, k)]	.108	.130	.112	.135	.301
se[s(i, j)-s(k, l)]	.099	.119	.102	.123	.245

weight accumulation S.C.A. value, whereas, the cross Ds3 x Ds4 showed equal dry weight accumulation and acetylene reduction S.C.A. values. These results suggested that dry weight accumulation and the acetylene reduction assay, although assumed to measure symbiotic nitrogen fixation, may measure closely related components of a complex character.

c. Experiment III.

Since both strain and/or environmental effects appeared to be of importance in the preceding studies, Experiment III was inititated to determine if strain X genotype interactions were of significant importance in a *D. sandwicense* population.

A review of the original and $\log_{10}$ transformed means for the within-strain and overall analyses for Experiment III illustrated that heterosis was of the approximate magnitude of 1.6 (Tables 32, 33 and 34; respectively). The low mean values for the acetylene reduction analysis in strain A (Table 32) appeared to be due to the nodules of that strain being in a state of senescence. Nodule senescence may have been due to the harsh "environmental" conditions imposed upon this experiment; yet strain B appeared unaffected. Since strain A was faster growing than strain B the nodules of strain A may have been sampled at a later physiological stage. The compatability of the dry weight means may be explained by the releasing of nitrogen from the decaying nodules for plant growth. Further

Table 32. Means and standard errors of the difference between two means for the Rhizobium strain A inoculated blocks for Experiment III.

	Dry weight (mg/pl)		Acetylene reduced (nM/pl/hr)	
Genotype	original	$\log_{10}$	original	$\log_{10}$
Ds1	139	2.045	4.3	0.436
Ds2	223	2.284	5.7	0.650
Ds3	167	2.187	6.3	0.714
Ds4	180	2.115	9.9	0.476
Ds5	225	2.277	11.2	0.845
Ds1 x Ds2	249	2.396	6.7	0.774
Ds1 x Ds3	309	2.481	10.0	0.982
Ds1 x Ds4	211	2.314	5.9	0.740
Ds1 x Ds5	363	2.556	16.5	1.214
Ds2 x Ds3	82	1.876	1.7	0.188
Ds2 x Ds4	302	2.454	8.1	0.877
Ds2 x Ds5	248	2.361	8.3	0.712
Ds3 x Ds4	173	2.226	3.5	0.497
Ds3 x Ds5	375	2.556	15.6	1.067
Ds4 x Ds5	415	2.572	22.2	1.124
se of d.	75	0.167	3.8	0.207

Table 33. Means and standard error of the difference between two means for the *Rhizobium* strain B inoculated blocks for Experiment III.

Genotype	Dry weight (mg/pl)		Acetylene reduced (nM/pl/hr)	
	original	$\log_{10}$	original	$\log_{10}$
Ds1	205	2.270	18.0	1.219
Ds2	223	2.335	15.9	1.197
Ds3	177	2.111	12.5	0.819
Ds4	161	2.184	9.5	0.923
Ds5	169	2.187	13.1	1.021
Ds1 x Ds2	260	2.404	24.1	1.340
Ds1 x Ds3	493	2.690	36.5	1.551
Ds1 x Ds4	182	2.235	15.0	1.160
Ds1 x Ds5	347	2.500	25.1	1.326
Ds2 x Ds3	351	2.542	29.6	1.453
Ds2 x Ds4	161	2.121	15.1	1.037
Ds2 x Ds5	325	2.491	31.3	1.468
Ds3 x Ds4	302	2.473	21.1	1.317
Ds3 x Ds5	287	2.439	19.4	1.267
Ds4 x Ds5	329	2.493	12.8	1.099
se of d.	67	0.155	6.9	0.203

Table 34. Means and standard error of the difference between two means from the overall analysis for Experiment III.

Genotype	Dry weight (mg/pl) original	Dry weight (mg/pl) $\log_{10}$	Acetylene reduced (nM/pl/hr) original	Acetylene reduced (nM/pl/hr) $\log_{10}$
Ds1	172	2.158	11.1	0.827
Ds2	223	2.310	10.8	0.923
Ds3	172	2.149	9.4	0.767
Ds4	170	2.150	9.7	0.699
Ds5	197	2.232	12.1	0.933
Ds1 x Ds2	254	3.400	15.4	1.057
Ds1 x Ds3	401	2.585	23.2	1.266
Ds1 x Ds4	196	2.274	10.5	0.950
Ds1 x Ds5	355	2.528	20.8	1.270
Ds2 x Ds3	216	2.209	15.7	0.820
Ds2 x Ds4	231	2.288	11.6	0.957
Ds2 x Ds5	287	2.426	19.8	1.090
Ds3 x Ds4	238	2.349	12.3	0.907
Ds3 x Ds5	331	2.498	17.5	1.167
Ds4 x Ds5	372	2.532	17.5	1.112
se of d.	50	0.114	3.9	0.145

experimentation would be necessary to clarify these results.

The within-strain diallel analyses of variance for the normal characters are presented in Table 35 and for comparison the $\log_{10}$ transformed diallel analyses of variance is given in Table 36. The similarity of the two scales suggested that scale effects had been removed from this experiment through improved experimental and bottle jar design.

Significant variation was again found among the genotypes and the majority of this variation was due to average heterosis. Block X genotype interactions were also found to be significant for must genetic components suggesting genetic component X environment interactions. These results were expected since Vincent (1965) reported that the environment influenced symbiotic nitrogen fixation.

Factorial diallel analyses of variance for original and $\log_{10}$ transformed characters are presented in Table 37. Strains were not significantly different for dry weight accumulation yet highly significant for the acetylene reduction assay for both scales due to nodule senescence in Strain A. Genotypes were highly significant for all characters and scales suggesting that genetic variation was present in this population and that this variation was scale independent.

A partition of the genotype variance suggested that a predominant portion of this variance was due to dominance deviations since general effects were significant for the

Table 35. Mean squares from the diallel analysis of variance for the original characters for Experiment III.

Source	df	Rhizobium Strain A Dry weight (mg/pl)	 Acetylene reduced (nM/pl/hr)	Rhizobium Strain B Dry weight (mg/pl)	 Acetylene reduced (nM/pl/hr)
Blocks	1	88167**	835**	31236*	858**
Genotypes	14	34999**	125**	36155**	250**
General	4	29706**	212**	20009**	282**
Heterosis	10	37116**	90**	42614**	237**
Average	1	98155**	67*	181352**	988**
Line	4	30939**	98**	25967**	154*
Specific	5	29850**	88**	28183**	154*
Blocks X genotypes	14	19993**	113**	16847**	88*
Blocks X general	4	17608*	171**	3580	92
Blocks X heterosis	10	20946**	91**	22153**	106*
Blocks X average	1	38593*	26	914	4
Blocks X line	4	8820	145**	26901**	157*
Blocks X specific	5	27119**	148**	22605**	47
Error	29	5671	14	4478	47

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 36. Mean squares from the diallel analysis of variance for the $\log_{10}$ transformed characters for Experiment III.

Source	df	Rhizobium Strain A Dry weight (mg/pl)	 Acetylene reduced (nM/pl/hr)	Rhizobium Strain B Dry weight (mg/pl)	 Acetylene reduced (nM/pl/hr)
Blocks	1	.410**	2.943**	.204*	.897**
Genotypes	14	.163**	.313**	.121**	.176**
General	4	.102*	.348**	.047	.170**
Heterosis	10	.187**	.299**	.150**	.178**
Average	1	.520**	.468**	.653**	.838**
Line	4	.161**	.323**	.121**	.132*
Specific	5	.141**	.248**	.073*	.082
Blocks X genotypes	14	.075*	.233**	.072**	.108*
Blocks X general	4	.034	.152*	.039	.129*
Blocks X heterosis	10	.090**	.265**	.085**	.099*
Blocks X average	1	.473**	.703**	.001	.093
Blocks X line	4	.046	.228**	.146**	.178**
Blocks X specific	5	.049	.207**	.053	.037
Error	29	.028	.043	.024	.041

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 37. Mean squares from a factorial diallel analysis of variance for Experiment III.

		Original		Log_{10}	
Source	df	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)
Strains	1	12523	342690**	.080	6.314**
Blocks	1	112180**	169254**	.596**	3.546**
Strains X blocks	1	7223	8	.018	.295
Genotypes	14	47483**	14407**	.178**	.227**
General	4	21909	11281	.079	.219*
Heterosis	10	57714**	15675**	.217**	.230**
Average	1	273652**	78416**	1.168**	1.279**
Line	4	36878**	9337	.147*	.156
Specific	5	31195**	8154	.083	.080
Strains X genotypes	14	23803*	23069**	.105**	.262**
Strains X general	4	27904*	38025*	.069	.298*
Strains X heterosis	10	22163*	17027**	.120**	.247**
Strains X average	1	6263	26956*	.004	.027
Strains X line	4	20118	15886*	.135*	.298*
Strains X specific	5	26978*	16055*	.131*	.250*
Error	86	9420	5324	.041	.084

*significant at the 5% level of probability.
**significant at the 1% level of probability.

$\log_{10}$ transformed acetylene reduction assay only. The additive effects which were present in the within-strain analyses may be cancelled in a factorial analysis (Comstock, R.E., pers. comm.). Average heterosis was highly significant for all analyses while the significance of line and specific heterosis was character and scale dependent.

Strain by genotype interactions were found to be significant in this experiment. Strain X heterosis and strain X specific heterosis interactions were significant for all analyses while there appeared to be little strain X average heterosis interaction.

A limitation to this analysis is the limited number of degrees of freedom for the interaction terms. Since strains have been considered to be an environmental component in this study, the strains and blocks were confounded and analysed as a four block experiment so that direct comparisons could be made with Experiment II (Table 28). The overall diallel analyses of variance for the original and $\log_{10}$ transformed characters are presented in Table 38. Genotypes were found to be significantly different for both scales as were all components with the exception of specific heterosis for the $\log_{10}$ transformed acetylene reduction assay. All interaction terms were significant with the exception of blocks/strains X general which was non-significant for the $\log_{10}$ transformed dry weight accumulation. The significance of the interaction terms suggested that genetic studies for total symbiotic nitrogen

Table 38. Mean squares from the overall diallel analysis of variance for Experiment III.

Source	df	Original		Log₁₀	
		Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)
Blocks/strains (B/s)	3	44024**	1793**	.231**	3.385**
Genotypes	14	47406**	156**	.178**	.227**
General	4	21800**	128**	.079*	.219**
Heterosis	10	57648**	168**	.217**	.230**
Average	1	273173**	893**	1.169**	1.279**
Line	4	36721**	100*	.147**	.156**
Specific	5	31286**	76*	.083*	.080
B/s x genotypes	42	20196**	143**	.084**	.201**
B/s x general	12	16336*	203**	.047	.193**
B/s x heterosis	30	21768**	120**	.098**	.204**
B/s x average	3	15280*	113**	.159**	.274**
B/s x line	12	18635**	124**	.109**	.234**
B/s x specific	15	25491**	117**	.078**	.165**
Error	58	5075	30	.026	.042

*significant at the 5% level of probability.
**significant at the 1% level of probability.

fixed should be tested with different strains and in different environments if the experimental results are to be reliable.

The error mean squares for dry weight accumulation and the acetylene reduction assay in Experiment III (Table 38) have been reduced by approximately one-half those of Experiment II (Table 28). This reduction is most likely due to the modification of the bottle jar design (Figure 3) which reduced within-cell variation. Once the variation due to bottle jar design was removed, the $\log_{10}$ transformation was not necessary as the original analyses were similar to the $\log_{10}$ analyses. These data suggest that further investigation of the design of bottle jars or sand size would be beneficial.

The estimates of the variety constants and G.C.A. effects for the original and $\log_{10}$ transformed characters from all analyses are presented in Tables 39 and 40, respectively. The estimates of the G.C.A. effects for the $\log_{10}$ transformed characters for strain B (Table 39) were found to be similar to the results of Experiment I. Line Ds1 was found to have the largest G.C.A. effect for both characters while Ds4 showed the largest negative G.C.A. effect. Line Ds2 showed the largest heterosis constant for dry weight but Ds1 had the larger constant for the acetylene reduction assay. The G.C.A. effects for strain A (Table 39) were not similar to those reported for Experiment II. As earlier discussed the nodules of the plants of strain A were

Table 39. Estimates of G.C.A. effects and variety constants for the original characters for Experiment III.

	Rhizobium Strain A		Rhizobium Strain B		Overall	
	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)
Griffing's G.C.A. effects						
g(Ds1)	-8	-1.0	15	2.5	3	0.8
g(Ds2)	-20	-2.6	-7	1.8	-13	-0.4
g(Ds3)	-27	-1.6	28	1.7	1	0.1
g(Ds4)	-1	.7	-42	-5.2	-21	-2.3
g(Ds5)	55	4.4	6	-0.7	30	1.8
se[g(i)]	13	.6	11	1.1	9	0.7
se[g(i)-g(j)]	20	1.0	18	1.8	13	1.0
Gardner's variety constants						
mean	187	7.5	187	13.8	187	10.6
v(Ds1)	-48	-3.2	18	4.2	15	0.5
v(Ds2)	36	-1.8	36	2.1	36	0.2
v(Ds3)	-20	-1.2	-10	-1.3	-15	-1.2
v(Ds4)	-7	2.4	-26	-4.3	-16	-0.9
v(Ds5)	38	3.7	-18	-0.7	10	1.5

Table 40. Estimates of G.C.A. effects and variety constants for the $\log_{10}$ transformed characters for Experiment III.

	Rhizobium Strain A		Rhizobium Strain B		Overall	
	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)
Griffing's G.C.A. effects						
g(Ds1)	-.006	.009	.026	.077	.010	.043
g(Ds2)	-.032	-.095	.006	.059	-.013	-.018
g(Ds3)	-.052	-.051	.025	-.008	-.014	-.029
g(Ds4)	-.012	-.047	-.071	-.117	-.042	-.082
g(Ds5)	.103	-.184	.015	-.011	.059	.087
se[g(i)]	.028	.036	.026	.032	.019	.024
se[g(i)-g(j)]	.044	.058	.042	.050	.030	.038
Gardner's variety constants						
mean	2.182	0.624	2.217	1.036	2.200	0.830
v(Ds1)	-.137	-.188	.053	.183	-.042	-.003
v(Ds2)	.102	.026	.118	.162	.110	.094
v(Ds3)	.006	.090	-.107	-.217	-.051	-.063
v(Ds4)	-.067	-.148	-.033	-.113	-.050	-.131
v(Ds5)	.096	.221	-.031	-.015	.032	.103

in a state of senescence and this physiological state may have affected the results for the acetylene reduction analysis. However the G.C.A. effects for dry weight accumulation were similar, therefore nodule senescence may not explain the divergence from the G.C.A. effects of Experiment II. Line Ds5 presented the largest G.C.A. effect for strain A. Its crosses had larger means than the other crosses (Table 32). Line Ds5 and its crosses may have been resistant to nodule senescense or responded more quickly to clipping of the juvenile plants. Line Ds5 also has the largest G.C.A. effect for both characters for the overall analysis (Table 39).

The estimates of the heterosis constants and the S.C.A. effects derived from the original and the $\log_{10}$ characters in the various analyses are presented in Tables 41 to 44, respectively.

d. Combined analysis.

The preceding experiments were conducted with the same diallel cross but differed in environmental and strain effects. Since strains have been initially considered as an environmental effect, it would be pertinent to pool the above experiments for a combined analysis. Blocks 1,2 and 3 of Experiment I have been deleted from the combined analysis because subsamples within cells were not available for block 1 and the acetylene reduction assay was not recorded for blocks 2 and 3. The remaining nine blocks, six inoculated

Table 41. Estimates of Gardner's average, line and specific heterosis constants for the original characters for Experiment III.

Heterosis constant	*Rhizobium* Strain A Dry weight (mg/pl)	*Rhizobium* Strain A Acetylene reduced (nM/pl/hr)	*Rhizobium* Strain B Dry weight (mg/pl)	*Rhizobium* Strain B Acetylene reduced (nM/pl/hr)	Overall Dry weight (mg/pl)	Overall Acetylene reduced (nM/pl/hr)
h(ave)	86	2.4	117	9.2	101	5.8
h(Ds1)	38	1.5	13	0.8	26	1.2
h(Ds2)	-88	-4.0	-57	1.6	73	-1.2
h(Ds3)	-41	-2.3	78	5.5	19	1.6
h(Ds4)	7	-1.1	-67	-7.2	-30	-4.2
h(Ds5)	85	5.9	34	-0.8	59	2.5
h(Ds1,Ds2)	33	1.8	-27	-4.5	3	-1.4
h(Ds1,Ds3)	73	3.1	84	5.7	84	4.4
h(Ds1,Ds4)	-79	-4.0	-64	-1.6	-71	-2.8
h(Ds1,Ds5)	-27	-1.0	-3	0.3	-15	-0.3
h(Ds2,Ds3)	71	-0.4	14	-1.0	-28	-0.7
h(Ds2,Ds4)	96	3.0	-23	-1.2	36	0.9
h(Ds2,Ds5)	-58	-4.5	36	6.7	-11	1.1
h(Ds3,Ds4)	-52	-3.6	6	2.5	-23	-0.5
h(Ds3,Ds5)	49	0.9	-114	-7.3	-32	-3.6
h(Ds4,Ds5)	36	4.5	81	0.3	58	2.4

Table 42. Estimates of Gardner's average, line and specific heterosis constants for the $\log_{10}$ transformed characters for Experiment III.

Heterosis constant	*Rhizobium* Strain A Dry weight (mg/pl)	*Rhizobium* Strain A Acetylene reduced (nM/pl/hr)	*Rhizobium* Strain B Dry weight (mg/pl)	*Rhizobium* Strain B Acetylene reduced (nM/pl/hr)	Overall Dry weight (mg/pl)	Overall Acetylene reduced (nM/pl/hr)
h(ave)	.198	.193	.221	.266	.209	.230
h(Ds1)	.145	.241	-.002	-.035	.072	.103
h(Ds2)	-.194	-.253	-.125	-.050	-.160	-.151
h(Ds3)	-.128	-.224	.183	.235	.027	.006
h(Ds4)	.050	.064	-.128	-.142	-.039	-.039
h(Ds5)	.128	.172	.071	-.008	.100	.082
h(Ds1,Ds2)	.083	.050	.006	-.049	.045	.000
h(Ds1,Ds3)	.151	.197	.097	.066	.124	.131
h(Ds1,Ds4)	-.158	-.214	-.084	.000	-.121	-.107
h(Ds1,Ds5)	-.076	-.032	-.020	-.016	-.048	-.024
h(Ds2,Ds3)	-.234	-.211	.039	-.006	-.097	-.109
h(Ds2,Ds4)	.202	.310	-.108	-.097	.047	.107
h(Ds2,Ds5)	.051	-.148	.063	.152	.006	.002
h(Ds3,Ds4)	-.044	-.131	.049	.087	.003	.022
h(Ds3,Ds5)	.127	.146	-.186	-.146	-.029	.000
h(Ds4,Ds5)	.000	.035	.143	.011	.071	.023

Table 43. Estimates of Griffing's S.C.A. effects for the original characters for Experiment III.

S.C.A. effect	Rhizobium Strain A		Rhizobium Strain B		Overall	
	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)
s(Ds1)	-89	-2.9	-89	-6.8	-89	-4.9
s(Ds2)	19	1.8	-29	-7.6	-5	-2.9
s(Ds3)	-22	.4	-144	-10.9	-83	-5.3
s(Ds4)	-63	-.6	-20	0.0	-41	-.3
s(Ds5)	-130	-6.6	-107	-5.5	-118	-6.0
se[s(i)]	26	1.3	23	2.3	17	1.3
se[s(i)-s(j)]	35	1.7	31	3.1	23	1.8
s(Ds1,Ds2)	32	1.2	-13	-0.1	10	0.6
s(Ds1,Ds3)	100	3.4	185	12.4	143	7.9
s(Ds1,Ds4)	-25	-3.0	-56	-2.1	-41	-2.5
s(Ds1,Ds5)	72	4.1	62	3.4	67	3.7
s(Ds2,Ds3)	-116	-3.2	64	6.2	-26	1.5
s(Ds2,Ds4)	78	0.9	-55	-1.3	11	-0.2
s(Ds2,Ds5)	-31	-2.6	62	10.3	15	3.9
s(Ds3,Ds4)	-43	-4.8	51	4.7	4	-0.1
s(Ds3,Ds5)	103	3.7	-11	-1.5	46	1.1
s(Ds4,Ds5)	116	8.0	100	-1.2	108	3.4
se[s(i,j)]	33	1.7	29	2.9	22	1.7
se[s(i,j)-s(i,k)]	49	2.5	44	4.4	33	2.5
se[s(i,j)-s(k,l)]	45	2.3	40	4.0	30	2.3

Table 44. Estimates of Griffing's S.C.A. effects for the $\log_{10}$ transformed characters for Experiment III.

S.C.A. effect	*Rhizobium* Strain A Dry weight (mg/pl)	*Rhizobium* Strain A Acetylene reduced (nM/pl/hr)	*Rhizobium* Strain B Dry weight (mg/pl)	*Rhizobium* Strain B Acetylene reduced (nM/pl/hr)	Overall Dry weight (mg/pl)	Overall Acetylene reduced (nM/pl/hr)
s(Ds1)	-.256	-.335	-.146	-.147	-.201	-.241
s(Ds2)	.035	.088	-.041	-.134	-.003	-.023
s(Ds3)	.022	.063	-.304	-.379	-.163	-.158
s(Ds4)	-.175	-.183	-.038	-.056	-.106	-.120
s(Ds5)	-.242	-.276	-.209	-.171	-.225	-.223
se[s(i)]	.057	.074	.054	.065	.039	.049
se[s(i)-s(j)]	.077	.100	.072	.087	.053	.066
s(Ds1,Ds2)	.121	.107	.008	-.009	.064	.049
s(Ds1,Ds3)	.226	.271	.275	.269	.250	.027
s(Ds1,Ds4)	.019	.025	-.084	-.013	-.033	.006
s(Ds1,Ds5)	.146	.268	-.094	.048	.120	.158
s(Ds2,Ds3)	-.353	-.419	.146	.188	-.103	-.115
s(Ds2,Ds4)	.185	.266	-.178	-.118	.003	.074
s(Ds2,Ds5)	-.023	-.130	.106	.207	.041	.039
s(Ds3,Ds4)	-.023	-.158	.154	.229	.066	.035
s(Ds3,Ds5)	.193	.181	.034	.072	.113	.126
s(Ds4,Ds5)	.168	.234	.184	.014	.176	.124
se[s(i,j)]	.072	.094	.068	.082	.050	.063
se[s(i,j)-s(i,k)]	.109	.141	.102	.124	.075	.094
se[s(i,j)-s(k,l)]	.099	.129	.093	.113	.068	.086

with strain A and three with strain B, were pooled such that block and strain effects were confounded.

The original and $\log_{10}$ means confirmed that the mean of the F_1 genotypes was much larger than the means of their inbred parents (Table 45). The mean heterosis for all characters appeared to be approximately 1.8.

The diallel analyses of variance for Gardner's Analysis II of the original and $\log_{10}$ transformed characters of the combined analysis are presented in Table 46 and for comparison Gardner's Analysis III is provided in Table 47. Both analyses are compatible; however, the general mean square was larger for Analysis III than for Analysis II suggesting that heritable effects are given more weighting by the Analysis III. Gardner and Eberhart (1966) report that Analysis III may be superior when average heterosis is highly significant as in the present data; however, either analysis is applicable.

Significant differences among genotypes were found for all characters except the original acetylene reduction assay. These results were not unexpected since the acetylene reduction assay values in the earlier experiments were affected by the difficulty of removing the root intact from the bottle jar wick. Highly significant heterosis and average heterosis effects were also recorded. All interaction effects for the $\log_{10}$ transformed characters were significant with the exception of block/strains x average heterosis for the $\log_{10}$ transformed acetylene

Table 45. Means and standard error of the difference between two means for the combined diallel analysis.

	Original		Log_{10}	
Genotype	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)
Ds1	118	47	1.953	1.267
Ds2	137	25	1.986	1.214
Ds3	126	49	2.001	1.309
Ds4	126	44	1.947	1.122
Ds5	135	37	2.042	1.343
Ds1 x Ds2	181	55	2.162	1.440
Ds1 x Ds3	262	77	2.288	1.556
Ds1 x Ds4	204	72	2.182	1.419
Ds1 x Ds5	248	96	2.273	1.636
Ds2 x Ds3	160	55	2.077	1.352
Ds2 x Ds4	193	59	2.197	1.450
Ds2 x Ds5	219	101	2.264	1.622
Ds3 x Ds4	229	97	2.298	1.507
Ds3 x Ds5	262	86	2.353	1.619
Ds4 x Ds5	271	97	2.270	1.457
se of d.	42	35	0.093	0.117

Table 46. Mean squares from Gardner's Analysis II for the [illegible]ined diallel analysis.

		Original		Log$_{10}$	
Source	df	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)
Blocks/Strains (B/s)	8	237264**	267858**	1.891**	8.486**
Genotypes	14	55338**	10882*	.352**	.428**
General	4	19363**	5999	.124*	.332**
Heterosis	10	69730**	12843**	.443**	.464**
Average	1	535941**	91299**	3.761**	3.884**
Line	4	28983**	5229	.071	.040
Specific	5	9092	3236	.077	.119
B/s x genotypes	112	16338**	6722	.076**	.152**
B/s x general	32	12392	4133	.069**	.136**
B/s x heterosis	80	17915**	7754	.079**	.156**
B/s x average	8	22822**	23354**	.112*	.115
B/s x line	32	14566*	6269	.080**	.188**
B/s x specific	40	19615**	5834	.071**	.139**
Error	129	8099	5604	.039	.062

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 47. Mean squares from Gardner's Analysis III for the combined diallel analysis.

		Original		Log$_{10}$	
Source	df	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)
Blocks/Strains (B/s)	8	237264**	267858**	1.891**	8.486**
Genotypes	14	55338**	10882*	.352**	.428**
Varieties	4	1068	1652	.028	.135
Varieties vs crosses	1	535941**	91299**	3.761**	3.900**
Crosses	9	26057**	6049	.118**	.173**
General	4	47263**	9578	.167**	.238**
Specific	5	9092	3236	.077	.121
B/s x genotypes	112	16338**	6722	.076**	.152**
B/s x varieties	32	5308	2135	.061*	.152**
B/s x varieties vs crosses	8	22822**	23354**	.112**	.115
B/s x crosses	72	20520**	6913	.078**	.157**
B/s x general	32	21650**	8260	.087**	.175**
B/s x specific	40	19615**	5834	.072**	.141**
Error	129	8099	5604	.039	.062

*significant at the 5% level of probability.
**significant at the 1% level of probability.

reduction assay. These results suggested that genotype x environment interactions were of significant importance in the study of total symbiotic nitrogen fixed. Significant general effects for the $\log_{10}$ transformed characters for both diallel analyses suggested that heritable genetic variation was present.

The combining ability analysis for G.C.A. effects and variety constants are presented in Table 48. As indicated by the genotype means, line Ds5 was shown to have the largest G.C.A. effect and variety constant for the $\log_{10}$ transformed characters. Lines Ds2 and Ds4, on the other hand, had negative G.C.A. effects. Heterosis constants and S.C.A. effects for the two scales are presented in Tables 49 and 50, respectively. The average heterosis constant was much larger that the other heterosis constants suggesting that the major portion of the genetic variance is due to F_1 heterosis (Table 49). On the other hand, all inbred lines exhibited negative S.C.A. effects suggesting inbreeding depression (Table 50). Further studies would have to be undertaken to determine whether the large average heterosis effects were due to F_1 heterosis or inbreeding depression exibited by the inbred lines when grown in a nitrogen limiting environment.

The combined analysis indicated that total symbiotic nitrogen fixed had both heritable and non-heritable components of the genetic variance. The larger proportion of variance was contributed by the non-heritable portion

Table 48. Estimates of G.C.A. effects and variety constants for the combined analysis.

	Original		Log$_{10}$	
	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)
Griffing's G.C.A. effects				
g(Ds1)	-23	-0.8	-.015	.008
g(Ds2)	-17	-11.1	-.035	-.033
g(Ds3)	3	2.1	.015	.018
g(Ds4)	0	2.1	-.011	-.064
g(Ds5)	17	7.6	.047	.071
se[g(i)]	7	6.0	.016	.020
se[g(i)-g(j)]	11	9.4	.025	.031
Gardner's variety constants				
mean	128	40.9	1.986	1.251
v(Ds1)	-11	6.1	-.033	.016
v(Ds2)	9	-14.9	.000	-.037
v(Ds3)	-2	8.7	.016	.058
v(Ds4)	-2	3.9	-.039	-.129
v(Ds5)	6	-3.9	.056	.092

Table 49. Estimates of Gardner's average, line and specific heterosis constants for the combined analysis.

Heterois constant	Original		Log$_{10}$	
	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nM/pl/hr)
h(ave)	95	39.0	.250	.255
h(Ds1)	6	-9.0	.003	.001
h(Ds2)	-51	-8.4	-.082	-.035
h(Ds3)	8	-5.4	.016	-.025
h(Ds4)	3	0.4	.020	.001
h(Ds5)	33	22.3	.044	.058
h(Ds1,Ds2)	3	-2.6	.210	-.022
h(Ds1,Ds3)	31	4.6	.415	.038
h(Ds1,Ds4)	-22	-3.5	-.041	-.033
h(Ds1,Ds5)	-12	1.5	-.022	.017
h(Ds2,Ds3)	-24	-7.6	-.101	-.104
h(Ds2,Ds4)	14	-6.9	.042	.061
h(Ds2,Ds5)	6	17.1	.038	.066
h(Ds3,Ds4)	-3	16.0	.038	.060
h(Ds3,Ds5)	-4	-13.0	.022	.006
h(Ds4,Ds5)	10	-5.6	-.039	-.089

Table 50. Estimates of Griffing's S.C.A. effects for the combined analysis.

	original		log$_{10}$	
S.C.A effect	Dry weight (mg/pl)	Acetylene reduced (nm/pl/hr)	Dry weight (mg/pl)	Acetylene reduced (nm/pl/hr)
s(Ds1)	-68	-18.3	-.169	-.172
s(Ds2)	-20	-18.8	-.097	-.140
s(Ds3)	-70	-21.4	-.181	-.148
s(Ds4)	-66	-26.3	-.184	-.170
s(Ds5)	-91	-45.1	-.204	-.219
se[s(i)]	15	12.2	.032	.040
se[s(i)-s(j)]	20	16.3	.043	.054
s(Ds1,Ds2)	9	.5	.059	.045
s(Ds1,Ds3)	71	9.4	.135	.110
s(Ds1,Ds4)	15	4.6	.055	.053
s(Ds1,Ds5)	42	22.2	.089	.136
s(Ds2,Ds3)	-16	-2.4	-.056	-.053
s(Ds2,Ds4)	19	1.5	.090	.125
s(Ds2,Ds5)	27	38.0	.100	.163
s(Ds3,Ds4)	35	26.2	.142	.131
s(Ds3,Ds5)	51	9.7	.139	.109
s(Ds4,Ds5)	62	20.4	.081	.031
se[s(i,j)]	19	15.4	.041	.051
se[s(i,j)-s(i,k)]	28	23.1	.061	.077
se[s(i,j)-s(k,l)]	25	21.1	.056	.070

because of the expression of average heterosis. Crosses Ds4 x Ds5, Ds1 x Ds3 and/or Ds2 x Ds5 would be of significant interest for an intensive study since these crosses exhibited high mean yield and specific combining ability effects for at least one of the two characters.

e. Correlation between dry weight accumulation and the acetylene reduction assay.

A secondary objective of this study was to relate dry weight accumulation and the acetylene reduction assay as quantitative measure for total symbiotic nitrogen fixation. The correlation coefficients for these characters for the diallel experiments are presented in Table 51. The correlation coefficients were found to be highly significant with the exception of the unadjusted overall correlation. These data suggest that the acetylene reduction assay is compatible with dry weight accumulation for juvenile plants grown in bottle jars. A similarly large correlation (r = .89) was found by Lawn *et al.* (1974) for total nodule activity and shoot dry weight for shoot genotype means.

It may be concluded that the acetylene reduction assay would be suitable as a plant breeding tool for screening of juvenile genotypes into various classes for total nitrogen fixed. Since the acetylene reduction assay does not require nitrogen-free growth conditions this assay would be effective and reliable for screening plants grown under field conditions.

Table 51. Unadjusted and block adjusted correlation coefficients between dry weight (mg/pl) and acetylene reduced (nM/pl/hr) for the diallel experiments.

Experiment	Unadjusted "r"	Block Adjusted "r"*
I	.92	.86
II	.92	.83
III	.76	.83
Combined	.43	.85

*all block means in each character are adjusted to the grand mean for that character.

2. Cross between two inbred lines.

A second approach to the study of quantitative genetic variance is provided by the analysis of populations produced by a cross between two inbred lines. Through the analysis of first degree statistics, the cross between two inbred lines will provide information about epistatic interactions which cannot be obtained from a diallel cross. In this study of the inheritance of symbiotic nitrogen fixation the cross between two inbred lines has been employed as an alternate quantitative genetic design for the establishment and the partition of the genetic variance and to investigate the influence of epistasis in the expression of heterosis. Three experiments were conducted in this section.

a. Experiment IV.

Seven blocks were grown in this experiment, three blocks were deleted due to their uniqueness. Blocks 7 and 8 were grown in large bottom (2l) bottle jars while the remainder had small bottles (600ml). Block 5 was missing many of the B_1 population plants and was not considered to be complete. Because of poor germination within the B_1 population, six B_1 plants from the deleted blocks were fitted into the remaining blocks so as not to appreciably change the within block mean.

The original and $\log_{10}$ means revealed F_1 heterosis with the F_2 mean falling very near the midparent value suggesting the random segregation of alleles (Table 52). The backcross

Table 52. Means, standard deviations and coefficients of variation for Experiment IV.

		Dry weight (mg/pl)		Acetylene reduced (nM/pl/hr)	
Genotype	n	original	$\log_{10}(X)$	original	$\log_{10}(X+1)$
P_1(Da1)	22	42.5±17.5(41)	1.60±.17(11)	45.1±26.1(58)	1.67±.04(2)
P_2(Da1)	28	17.8± 7.6(43)	1.22±.17(14)	10.9± 7.9(72)	1.20±.19(16)
F_1	16	58.5±33.3(57)	1.82±.19(10)	52.0±27.7(53)	1.72±.20(12)
F_2	123	29.8±20.1(67)	1.38±.30(22)	30.6±29.4(96)	1.45±.32(22)
B_1	22	16.3± 9.8(60)	1.12±.33(29)	15.3±10.3(67)	1.29±.20(16)
B_2	37	23.1±12.4(54)	1.30±.25(19)	18.5±17.6(95)	1.32±.25(19)

means do not appear to fit any pattern.

The mean squares of the analysis of variance of population means for the original and $\log_{10}$ transformed data are presented in Table 53. The analysis of variance of the population means indicated the blocks were not significantly different while populations were highly significantly different. Additive, dominance and epistatic effects were also significant with dominance by dominance interactions accounting for the major epistatic variation. The test for non-additivity revealed that the $\log_{10}$ scale was the most appropriate due to the effects of the bottle jar design (discussed earlier in the diallel section).

b. Experiment V.

The means of the six populations created from a cross between Ds2 ans Dsp (Table 1) revealed that the F_1 mean was larger than the other means indicating the presence of F_1 heterosis in this population (Table 54). The backcross population means tended to exceed the F_1 mean which is unexpected for an additive model. These results suggest epistatic interactions. The mean for Dsp was found to be much less than expected. Observing the F_2 population distribution for the original and $\log_{10}$ dry weight accumulation it was noted that the $\log_{10}$ data was skewed toward the upper end of the distribution (Figure 11). These distributions may be interpreted as a confounding of two distributions (Figure 12). The phenomenon producing the low

Table 53. Mean squares from the genetic analysis of variance of population means for Experiment IV.

		Dry weight (mg/pl)		Acetylene reduced (nM/pl/hr)	
Source	df	original	$\log_{10}(X)$	original	$\log_{10}(X+1)$
Blocks	4	155.1	.007	104.8	.003
Populations	5	1370.5**	.260**	1467.8**	.227**
Additive	1	1033.0**	.186**	2339.4*	.434**
Dominance	1	1451.3**	.149**	1034.3*	.151*
Epistatis	3	1456.0**	.322**	1322.0**	.183**
Add-Add	1	330.9	.087*	600.0	.066
Add-Dom	1	731.9*	.262**	826.8*	.131*
Dom-Dom	1	3305.2**	.616**	2539.6**	.353**
Error	20	126.2	.014	187.6	.018
Non-additivity	1	1418.5**	.067	1267.5**	.002
Residual	19	58.2	.012	131.0	.019

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 54. Means, standard deviations and coefficients of variation for Experiment V.

		Dry weight (mg/pl)		Acetylene reduced (nM/pl/hr)	
Genotype	n	original	$\log_{10}(X)$	original	$\log_{10}(X+1)$
P_1(Ds2)	15	108±34(31)	2.01±.15(8)	53.6±21.1(39)	1.74±.17(10)
P_2(Dsp)	14	8± 7(88)	0.72±.34(47)	2.0± 2.0(99)	0.92±.08(9)
F_1	15	119±32(27)	2.06±.14(7)	71.1±19.6(28)	1.87±.11(6)
F_2	88	112±57(51)	1.96±.35(18)	64.0±41.2(64)	1.76±.29(16)
B_1	43	144±51(35)	2.12±.18(8)	86.1±35.1(41)	1.92±.19(10)
B_2	31	142±73(51)	2.04±.40(20)	78.1±45.0(58)	1.83±.32(17)

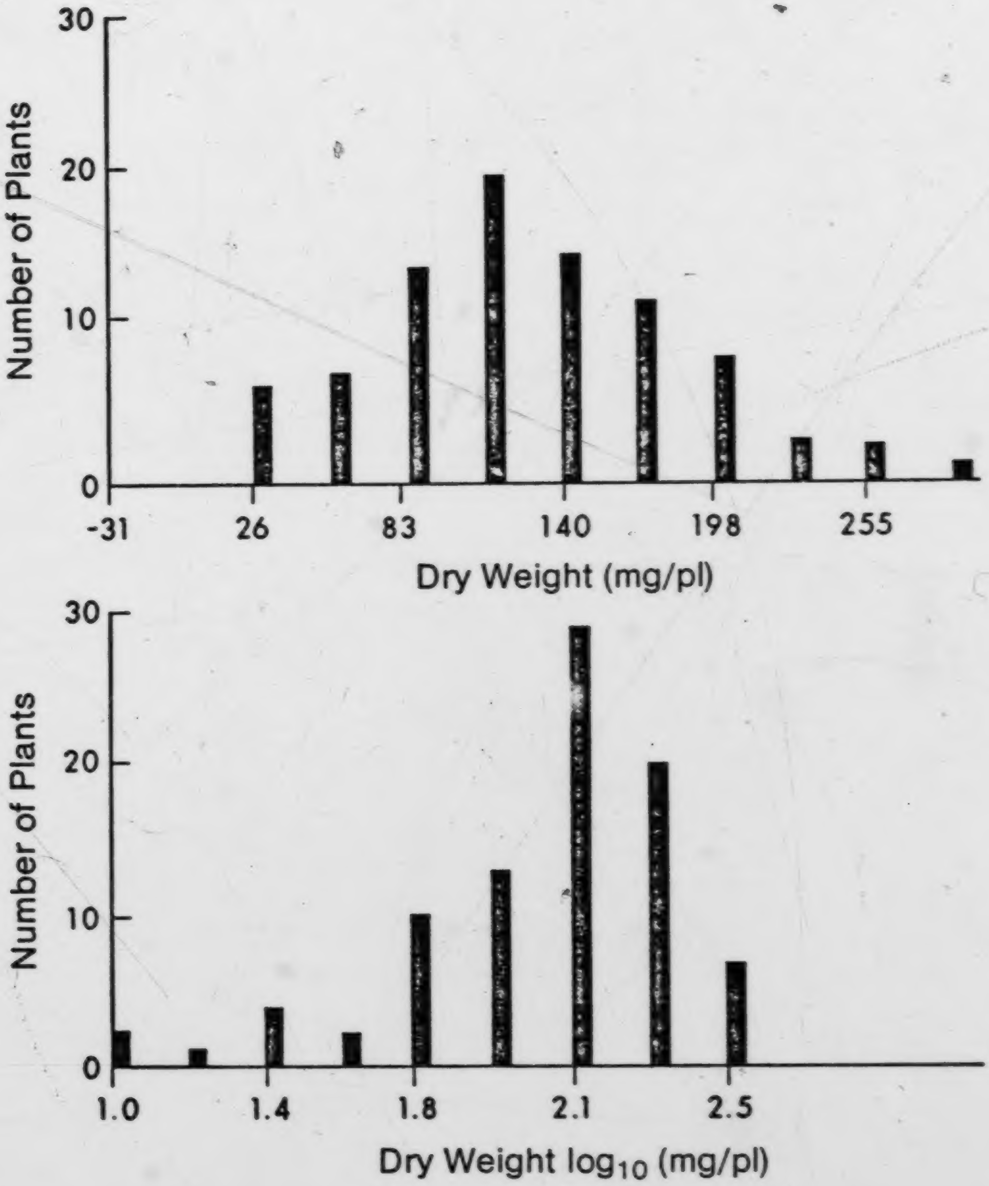

Figure 11. Normal and $\log_{10}$ distributions of the F_2 dry weight values for Experiment V.

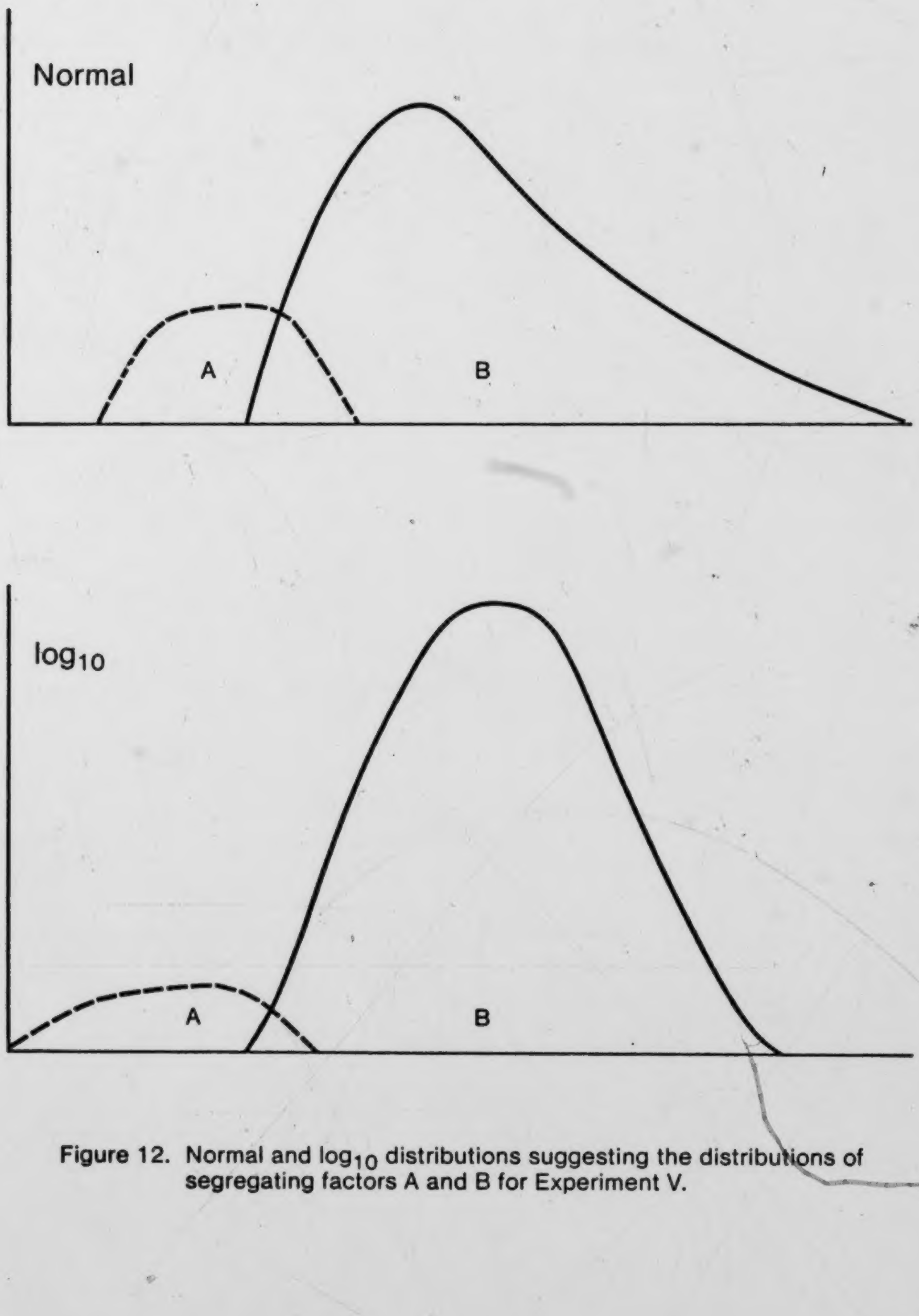

Figure 12. Normal and $\log_{10}$ distributions suggesting the distributions of segregating factors A and B for Experiment V.

Dsp, F_2 and B_2 scores appeared to be a gene segregating for pH effects since the Dsp line grew normally at pH 6.2±.2. Further investigation would be necessary to determine the exact nature of the low Dsp scores.

The six populations of a cross between Ds2 and Dsp when analysed for the analysis of variance of population means showed highly significant population differences (Table 55). Additive, dominance and epistatic effects were highly significant. Dominance X dominance and additive X dominance interactions contributed to epistatic variation. These results were compatible with those of Experiment IV.

c. Experiment VI.

The combined analysis of the diallel suggested that three crosses showed high mean yield and specific combining ability for the characters studied. Cross Ds2 x Ds5 was the cross which had the highest mean yield for the acetylene reduction assay so in Experiment VI the six populations of the Ds2 x Ds5 cross were created for intensive study.

The correlation cofficients for the characters studied are presented in Table 56. The estimates of N_2 fixation were found to be highly correlated among themselves and partially correlated with the leaf characters. Applying the principal components transformation to the fixation estimate characters yielded one factor (Table 57). The estimate variables were highly correlated with the first factor indicating that all estimates measure the principal axis of

Table 55. Mean squares from a genetic analysis of variance of population means for Experiment V.

Source	df	Dry weight (mg/pl)		Acetylene reduced (nM/pl/hr)	
		original	$\log_{10}(X)$	original	$\log_{10}(X+1)$
Blocks	4	1307*	.008	989	.017
Populations	5	12810**	1.450**	4690**	.708**
Additive	1	20664**	3.471**	5509**	1.482**
Dominance	1	21831**	2.295**	10567**	1.384**
Epistatis	3	7186**	.494**	2455**	.226**
Add-Add	1	2945*	.054	1424	.054
Add-Dom	1	4892**	.651**	1010	.222**
Dom-Dom	1	13721**	.776**	4926**	.402**
Error	20	384	.026	414	.015
Non-additivity	1	1080	.019	1246	.026
Residual	19	384	.026	372	.015

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 56. Correlations for Experiment VI.

Character		Correlations							
		1	2	3	4	5	6	7	8
Acetylene reduced	1	1.00							
Shoot fresh weight	2	0.83	1.00						
Root fresh weight	3	0.73	0.82	1.00					
Shoot dry weight	4	0.84	0.98	0.83	1.00				
Cotyledon length	5	0.19	0.29	0.36	0.28	1.00			
Cotyledon width	6	0.20	0.27	0.32	0.26	0.75	1.00		
Cotyledon area	7	0.21	0.31	0.37	0.29	0.93	0.94	1.00	
Juvenile leaf width	8	0.19	0.28	0.37	0.27	0.56	0.45	0.54	1.00

Table 57. Eigenvectors for factors from Experiment VI.

Character	Factor 1	2	3	4
Acetylene reduced	0.90	-.35	0.24	0.00
Top fresh weight	0.97	-.01	-.22	0.11
Root fresh weight	0.90	0.38	0.20	0.00
Top dry weight	0.97	-.02	-.20	-.11
Eigenvalue	3.51	0.27	0.19	0.02
% variation explained	88	7	5	0

variation; namely N_2 fixation. Shoot fresh and dry weight appeared to be the better estimates of N_2 fixation since they are most highly correlated with the principal axis. Since scores for the principal axis can be defined with a principal components transformation, the "factor" scores were calculated for the principal axis from the eigenvectors. The new derived 'character' has been termed the "Nitrogen fixation" factor and differences among population means are of importance rather than the amount of N_2 fixed. The "Nitrogen fixation" factor has been given a distribution of 1/3 the mean and standard deviation of the acetylene reduction assay since nitrogenase is assumed to theoretically reduce 1/3 the amount of N_2 than acetylene (Burris 1974). The "Nitrogen fixation" factor was added to the data set and treated as another character for the remaining analyses in Experiment VI.

The means and standard deviations for the original and $\log_{10}$ transformed data are presented in Tables 58 and 59, respectively. The F_1 mean was found to be greater than either parent for all scales and characters while the F_2 and backcross means were not significantly different from the parents. The Ds2 mean was found to be significantly smaller than the B_1 mean for the cotyledon characters (Table 58). Both the Ds2 and B_1 means were significantly less than the other population means suggesting a genetic basis to the difference. A similar pattern has also been shown for the juvenile leaf character.

Table 58. Means, standard deviations, and coefficients of variation for the original characters for Experiment VI.

Population	n	Acetylene reduced (nM/pl/hr)	Shoot fresh weight (mg/pl)	Root fresh weight (g/pl)	Shoot dry weight (mg/pl)	Nitrogen fixation "factor"
P_1(Ds2)	58	65±37(57)ab[+]	525±284(54)b	1.37±0.86(63)d	137±78(57)b	19±12(64)bc
P_2(Ds5)	55	51±22(43)b	475±170(36)b	1.66±0.63(38)b-d	121±47(39)b	17± 7(43)c
F_1	59	72±33(45)a	652±232(36)a	1.92±0.82(43)ab	173±64(37)a	24±10(41)a
F_2	229	59±30(52)b	537±219(41)b	1.73±0.85(49)bc	138±62(45)b	20±10(50)bc
B_1	108	58±30(52)b	522±243(47)b	1.49±0.82(55)cd	138±70(51)b	19±11(57)bc
B_2	111	63±35(56)ab	574±266(46)b	2.06±1.14(55)a	149±73(49)b	22±12(55)ab

[+] means followed by the same letter are not significantly different for the Student-Newman-Keul range test.

Table 58 (cont). Means, standard deviations, and coefficients of variation for the original characters for Experiment VI.

Population	n	Cotyledon Length (mm)	Cotyledon Width (mm)	Cotyledon Area (mm^2)	Juvenile leaf width (mm)
P$_1$(Ds2)	58	6.8±.5(7)c^{+}	4.3±.5(12)c	29±5(17)c	5.7±0.6(11)d
P$_2$(Ds5)	55	8.0±.7(9)a	5.0±.5(10)a	40±7(17)a	7.4±0.9(12)a
F$_1$	59	8.2±.6(7)a	5.0±.4(8)a	41±5(12)a	7.1±0.6(8)ab
F$_2$	229	8.0±.7(9)a	4.9±.5(10)a	39±7(18)a	7.0±0.7(10)b
B$_1$	108	7.4±.7(9)b	4.5±.5(11)b	34±6(18)b	6.1±0.9(15)c
B$_2$	111	8.1±.9(11)a	5.0±.6(12)a	41±9(22)a	7.3±1.3(18)a

$^{+}$means followed by the same letter are not significantly different for the Student-Newman-Keul range test.

Table 59. Means, standard deviations, and coefficients of variation for the $\log_{10}$ transformed characters for Experiment VI.

Population	n	Acetylene reduced (nM/pl/hr)	Shoot fresh weight (mg/pl)	Root fresh weight (g/pl)	Shoot dry weight (mg/pl)	Nitrogen fixation "factor"
P_1(Ds2)	58	1.738±.273(10)ab+	2.663±.225(8)b	0.070±.245(350)c	1.068±.248(12)b	1.190±.040(34)b
P_2(Ds5)	55	1.666±.205(8)b	2.646±.174(7)b	0.182±.202(111)ab	1.046±.190(9)b	1.182±.251(21)b
F_1	59	1.811±.212(7)a	2.782±.179(6)a	0.241±.196(81)a	1.202±.188(8)a	1.345±.212(16)a
F_2	229	1.713±.234(9)b	2.693±.186(7)b	0.187±.211(113)a	1.096±.203(10)b	1.242±.236(19)b
B_1	108	1.692±.275(10)b	2.673±.199(7)b	0.112±.231(206)bc	1.084±.223(11)b	1.203±.264(22)b
B_2	111	1.729±.268(10)ab	2.707±.226(8)b	0.243±.261(107)a	1.111±.248(12)b	1.265±.292(23)ab

+means followed by the same letter are not significantly different for the Student-Newman-Keul range test.

The analyses of variance of the population means for the original and the $\log_{10}$ transformed characters are given in Tables 60 and 61, respectively. Populations and dominance effects were highly significant for all characters in both scales; however, additive effects were significant for root fresh weight. Epistasis appeared to be absent for most characters. These data suggest a completely non-heritable model for the estimates of total symbiotic nitrogen fixation.

The analyses of variance of the population means for the cotyledon and juvenile leaf characters indicated that an additive-dominance model adequately explains the distribution of the means. These data suggest that large cotyledons and juvenile leaves appear to be conditioned by a dominant allele at a single locus or a block of loci segregating as a single unit. To determine whether the cotyledon and juvenile leaf size expressed pleiotropism the cotyledon area and juvenile leaf width characters were subdivided into groups. The crosstabulation of the groups indicated that plants with small cotyledons and large juvenile leaves and *vice versa* were found implying that the cotyledon and juvenile leaf sizes are controlled by different genes (Table 62).

Having defined the cotyledon area and juvenile leaf width groups, it would be of interest to determine if plants with larger cotyledons and juvenile leaves would fix more nitrogen. The means, standard deviations, and coefficients

Table 60. Mean squares for the original characters for Experiment VI.

Source	df	Acetylene reduced (nM/pl/hr)	Shoot fresh weight (mg/pl)	Root fresh weight (g/pl)	Shoot dry weight (mg/pl)	Nitrogen fixation "factor"
Populations	5	3245**	227826**	5.58**	18687**	455**
Additive	1	3067	14997	11.23**	2648	2
Dominance	1	6757**	878971**	10.21**	73462**	1688**
Epistasis	3	2133	81914	2.16*	5772	110
Add-Add	1	98	4936	0.11	8490	195
Add-Dom	1	3994	152147	6.16**	8683	473
Add-Dom	1	2305	88657	0.21	143	1
Error	614	999	55668	0.79	4364	111

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 60 (cont). Mean squares for the original characters for Experiment VI.

Source	df	Cotyledonary leaf			Juvenile leaf width (mm)
		Length (mm)	Width (mm)	Area (mm^2)	
Populations	5	21.3**	6.4**	1668**	36.2**
Additive	1	73.2**	26.4**	6349**	159.2**
Dominance	1	29.6**	5.0**	1746**	15.4**
Epistasis	3	1.2	0.3	81	2.3*
Add-Add	1	1.4	0.1	73	0.8
Add-Dom	1	0.4	0.4	92	3.2*
Add-Dom	1	1.6	0.3	79	2.1
Error	614	0.5	0.3	47	0.8

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 61. Mean squares for the $\log_{10}$ transformed characters for Experiment VI.

Source	df	Acetylene reduced (nM/pl/hr)	Shoot fresh weight (mg/pl)	Root fresh weight (g/pl)	Shoot dry weight (mg/pl)	Nitrogen fixation "factor"
Populations	5	.155*	.143**	.376**	.178**	.239**
Additive	1	.072	.000	.983**	.002	.012
Dominance	1	.390*	.602**	.720**	.762**	.997**
Epistasis	3	.105	.036	.058	.042	.062
Add-Add	1	.000	.000	.006	.000	.002
Add-Dom	1	.132	.045	.170	.034	.111
Add-Dom	1	.182	.063	.001	.091	.074
Error	614	.061	.039	.051	.047	.066

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 62. Numbers of plants for cotyledon area by juvenile leaf width crosstabulation for Experiment VI.

	Juvenile leaf width			
Cotyledon area	< 6 mm	6-7 mm	> 7 mm	Row total
< 32 mm^2	96	48	12	156
32-39 mm^2	52	57	21	130
39-42 mm^2	35	91	49	175
> 42 mm^2	10	56	96	162
Column total	193	252	178	623

of variation for the nitrogen fixation estimates are presented in Table 63. Plants with larger cotyledons and juvenile leaves fixed significantly more nitrogen. It was also noted that plants growing in fine sand also fixed more nitrogen than did plants growing in very fine sand (Table 63). Combining the above groupings with populations, a factorial analysis of variance was performed on the estimates of nitrogen fixation to determine whether population interactions existed. These analyses of the original and $\log_{10}$ transformed data are presented in Tables 64 and 65, respectively. All main effects were highly significant for all estimates except root fresh weight for populations when adjusted for the other main effects. The only interaction of significance was population by sand size indicating that changing sand size in an experiment may affect the estimates of nitrogen fixation. Since cotyledon area and juvenile leaf width groups show significant differences for the total fixation estimates, it would be of interest to treat cotyledon area and juvenile leaf width as covariates to adjust the estimates for effects due to cotyledon or juvenile leaf size (juvenile plant vigor) because these leaves are functional during the onset of nodulation. Because plants with larger cotyledons and juvenile leaves may fix more nitrogen (Mytton, 1973), this advantage should be removed from the analysis to understand the mechanisms of N_2 fixation. The analysis of covariance for the estimates for the original and $\log_{10}$ transformed

Table 63. Means, standard deviations and coefficients of variation for plant groupings for Experiment VI.

Grouping	n	Acetylene reduced (nM/pl/hr)	Shoot fresh weight (mg/pl)	Root fresh weight (g/pl)	Shoot dry weight (mg/pl)	Nitrogen fixation "factor"
Cotyledon area						
< 32 mm^2	157	56±33(60)b$^+$	473±259(35)c	1.37±0.80(38)c	122±73(60)c	17±11(66)c
32-39 mm^2	128	55±24(44)b	487±192(39)c	1.50±0.68(45)c	126±54(43)c	18± 8(46)c
39-42 mm^2	175	62±32(53)b	563±221(39)b	1.80±0.86(48)b	146±64(44)b	21±10(48)b
> 42 mm^2	159	69±34(49)a	644±235(36)a	2.17±1.02(47)a	168±65(39)a	25±11(44)a
Juvenile leaf width						
< 6 mm	193	55±31(56)b$^+$	474±227(48)c	1.38±0.74(54)c	123±65(53)c	17±10(58)c
6-7 mm	250	59±31(53)b	546±239(44)b	1.69±0.83(49)b	141±67(48)b	20±10(52)b
> 7 mm	176	69±32(47)a	624±226(36)a	2.15±1.00(47)a	163±63(39)a	24±10(43)a
Sand size						
Very fine(.15mm)	313	56±27(47)b$^+$	505±204(40)b	1.70±0.91(54)ab	132±60(45)b	19±10(50)b
Mixed 1/2 each	155	68±38(55)a	563±273(48)a	1.87±1.03(55)a	149±77(52)a	22±12(57)a
Fine(.25mm)	153	62±34(55)a	609±251(41)a	1.62±0.76(47)b	152±68(45)a	21±11(50)a

$^+$means followed by the same letter are not significantly different for the Student-Newman-Keul range test.

Table 64. Mean squares from a factorial analysis of variance of the original characters for Experiment VI.

Source	df	Acetylene reduced (nM/pl/hr)	Shoot fresh weight (mg/pl)	Root fresh weight (g/pl)	Shoot dry weight (mg/pl)	Nitrogen fixation "factor"
Populations (P)	5	4997**	286721**	1.39	27477**	485**
Cotyledon group (C)	3	4235**	463163**	7.62**	36089**	930**
Juvenile leaf group (J)	2	4341**	395225**	6.37**	28700**	794**
Sand size (S)	2	7932**	516164**	4.93**	23475**	589**
P x C	14	793	35585	0.66	2538	75
P x J	9	1042	69065	1.29*	5481	144
P x S	10	1803*	131303**	1.30*	10174**	243**
C x J	6	503	43294	0.71	3574	86
C x S	6	1126	62469	0.91	3442	125
J x S	4	639	37881	0.45	2473	62
Error	556	885	45784	0.65	3678	92

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 65. Mean squares from a factorial analysis of variance of the $\log_{10}$ transformed characters for Experiment VI.

Source	df	Acetylene reduced (nM/pl/hr)	Shoot fresh weight (mg/pl)	Root fresh weight (g/pl)	Shoot dry weight (mg/pl)	Nitrogen fixation "factor"
Populations (P)	5	0.257**	0.198**	0.043	0.283**	0.269**
Cotyledon group (C)	3	0.236**	0.401**	0.534**	0.483**	0.672**
Juvenile leaf group (J)	2	0.334**	0.336**	0.464**	0.387**	0.555**
Sand size (S)	2	0.238**	0.268**	0.197**	0.158*	0.157
P x C	14	0.083	0.032	0.052	0.038	0.066
P x J	9	0.065	0.049	0.078	0.054	0.084
P x S	10	0.125*	0.099**	0.101**	0.118**	0.156**
C x J	6	0.005	0.017	0.038	0.018	0.024
C x S	6	0.051	0.044	0.051	0.038	0.067
J x S	4	0.010	0.014	0.010	0.011	0.009
Error	556	0.054	0.031	0.041	0.039	0.054

*significant at the 5% level of probability.
**significant at the 1% level of probability.

data are presented in Tables 66 and 67, respectively. As expected the covariates were highly significant as were differences among the populations (see Table 66). However, dominance deviations were no longer significant whereas additive effects have become highly significant. These data suggest that the "efficiency" (amount of fixation following nodule formation) of nitrogen fixation appears to be heritable. Examining the population means following adjustment for the covariates revealed that the Ds2 mean was adjusted upward (Table 68) as expected from earlier data (Table 60).

Since Ds2 was shown to be similar to Ds5 for the unadjusted estimate means and significantly different for cotyledon and juvenile leaf size it may be assumed that Ds2 is more "efficient" for total fixation per unit area of cotyledon or juvenile leaf than is Ds5.

A method to investigate this "efficiency" of fixation would be to divide the nitrogen fixation estimates by either the cotyledon area or juvenile leaf width to create characters of ratios of fixation per unit of cotyledon area or juvenile leaf width. The population means for the estimate ratios have been given in Tables 69 and 70 for cotyledon area and juvenile leaf width adjustment, respectively. For the estimate ratios, Ds2 was again found to be significantly more "efficient" than Ds5 for all estimates except root fresh weight. The analysis of variance for the estimate ratios revealed that total genetic effects

Table 66. Mean squares from an analysis of covariance of the original characters for Experiment VI.

Source	df	Acetylene reduced (nM/pl/hr)	Shoot fresh weight (mg/pl)	Root fresh weight (g/pl)	Shoot dry weight (mg/pl)	Nitrogen fixation "factor"
Covariates	2	15799**	1926033**	44.5**	132383**	4058**
Cotyledon area	1	10589**	1209806**	19.0**	80914**	2293**
Juvenile leaf width	1	4570*	619485**	22.5**	44374**	1517**
Populations	5	5831**	357361**	1.5*	33075**	605**
Additive	1	24088**	1469771**	3.7*	133226**	2441**
Dominance	1	628	136462	0.6	17143*	212
Epistasis	3	1480	60186	1.1	5002	125
Add-Add	1	2949	144609	1.0	11979	217
Add-Dom	1	1490	30737	2.2	2154	131
Dom-Dom	1	2	5212	0.1	872	26
Error	608	916	48042	0.7	3789	95

*significant at the 5% level of probability.

**significant at the 1% level of probability.

Table 67. Mean squares from an analysis of covariance of the $\log_{10}$ transformed characters for Experiment VI.

Source	df	Acetylene reduced (nM/pl/hr)	Shoot fresh weight (mg/pl)	Root fresh weight (g/pl)	Shoot dry weight (mg/pl)	Nitrogen fixation "factor"
Covariates	2	1.134**	1.666**	3.331**	1.850**	2.906**
Cotyledon area	1	0.788**	1.079**	1.683**	1.215**	1.839**
Juvenile leaf width	1	0.308*	0.511**	1.426**	0.554**	0.924**
Populations	5	0.303**	0.240**	0.041	0.334**	0.308**
Additive	1	1.266**	1.060**	0.164*	1.392**	1.345**
Dominance	1	0.010	0.059	0.020	0.131	0.053
Epistasis	3	0.080	0.017	0.007	0.049	0.047
Add-Add	1	0.008	0.000	0.015	0.144	0.139
Add-Dom	1	0.026	0.050	0.006	0.002	0.002
Dom-Dom	1	0.205	0.000	0.000	0.000	0.000
Error	608	0.055	0.032	0.041	0.039	0.052

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 68. Adjusted means from an analysis of covariance for Experiment VI.

Population	n	Acetylene reduced (nm/pl/hr)	Shoot fresh weight (mg/pl)	Root fresh weight (g/pl)	Shoot dry weight (mg/pl)	Nitrogen fixation "factor"
		original				
P_1(Ds2)	58	78.0	657	1.88	173	24.9
P_2(Ds5)	55	47.2	432	1.47	106	15.4
F_1	59	68.7	617	1.78	164	23.0
F_2	229	57.1	519	1.67	134	19.0
B_1	108	65.0	596	1.77	158	22.1
B_2	111	58.1	521	1.85	135	20.3
		$\log_{10}$				
P_1(Ds2)	58	1.85	2.79	0.21	2.20	1.35
P_2(Ds5)	55	1.64	2.61	0.14	2.00	1.15
F_1	59	1.78	2.75	0.20	2.17	1.30
F_2	229	1.70	2.68	0.17	2.08	1.22
B_1	108	1.75	2.75	0.19	2.15	1.29
B_2	111	1.68	2.66	0.19	2.06	1.20

Table 69. Means, standard deviations, and coefficients of variation for N fixation estimates/cotyledon area for Experiment VI.

Population	n	Acetylene reduced ratio	Shoot fresh weight ratio	Root fresh weight ratio	Shoot dry weight ratio	Nitrogen fixation "factor" ratio
P_1(Ds2)	58	2.25±1.35(60)a	18.2±10.2(56)a	.047±.030(64)a	4.74±2.82(59)a	.659±.437(66)a
P_2(Ds5)	55	1.29±0.57(44)c	11.7± 3.8(32)c	.041±.015(37)a	2.98±1.04(35)c	.429±.176(41)c
F_1	59	1.78±0.80(45)b	16.0± 5.6(40)ab	.047±.021(45)a	4.25±1.52(36)ab	.602±.243(40)ab
F_2	229	1.52±0.76(50)bc	13.8± 5.6(41)bc	.044±.021(48)a	3.54±1.57(44)c	.509±.249(49)bc
B_1	108	1.75±0.97(55)b	15.9± 8.0(50)ab	.045±.025(56)a	4.18±2.22(53)ab	.570±.333(58)ab
B_2	111	1.53±0.77(50)bc	13.9± 5.7(41)bc	.049±.024(49)a	3.59±1.62(45)bc	.529±.260(49)bc

[+] means followed by the same letter are not significantly different for the Student-Newman-Keul range test.

Table 70. Means, standard deviations, and coefficients of variation for N fixation estimates/juvenile leaf width for Experiment VI.

Population	n	Acetylene reduced ratio	Shoot fresh weight ratio	Root fresh weight ratio	Shoot dry weight ratio	Nitrogen fixation "factor" ratio
P_1(Ds2)	58	11.4±6.2(54)a	91.8±45.8(50)a	.24±.15(63)a	23.9±12.9(54)a	3.33±2.03(61)a
P_2(Ds5)	55	7.0±2.7(39)c	64.7±19.4(30)c	.23±.08(35)a	16.4± 5.4(33)d	2.36±0.88(37)b
F_1	59	10.2±4.7(46)ab	91.8±31.2(35)a	.27±.11(41)a	24.3± 8.6(35)a	3.45±1.35(39)a
F_2	229	8.5±4.4(52)bc	77.2±31.5(41)b	.25±.12(48)a	19.8± 8.8(44)c	2.85±1.41(49)a
B_1	108	9.5±4.9(52)b	85.7±38.3(45)ab	.24±.13(54)a	22.6±11.0(49)ab	3.08±1.68(55)a
B_2	111	8.6±4.5(52)bc	78.0±33.6(43)ab	.27±.14(52)a	20.2± 9.5(47)bc	2.98±1.52(51)a

⁺means followed by the same letter are not significantly different for the Student-Newman-Keul range test.

were additive for cotyledon adjusted data (Table 71) whereas dominance effects were shown to be present for shoot weights and the "Nitrogen fixation" factor for the juvenile leaf adjusted data (Table 72). Grouping the shoot dry weight estimate ratios and crosstabulating with either cotyledon area or juvenile leaf groups, revealed the presence of plants with large cotyledons or juvenile leaves with "efficiency" of fixation and vice versa (Tables 73 and 74). It would appear to be possible to select plants with vigorous seedlings which were "efficient" for fixation.

D. Host genes for symbiotic nitrogen fixation in Desmodium.

The genes controlling the formation of nitrogenase are found on the Rhizobium genome (Bergerson, 1974). Since each experiment was inoculated with what was assumed to be a homogeneous Rhizobium culture then the nitrogenase enzyme should be uniform within each experiment. Significant genotype effects may indicate that the host plants differed in their ability to support nitrogen fixation physiologically. Nitrogenase would not be expected to be the limiting factor since it was expected to be the same for the "efficient" as for the "inefficient" genotypes. It must be concluded that there are genes within Desmodium which may indirectly regulate nitrogen fixation. Increasing the fixation rate of the Desmodium - Rhizobium interaction may not be completely effective by solely manipulating the Rhizobium strains which has been the traditional practice.

Table 71. Mean squares for the N fixation estimates/cotyledon area for Experiment VI.

Source	df	Acetylene reduced ratio	Shoot fresh weight ratio	Root fresh weight ratio	Shoot dry weight ratio	Nitrogen fixation "factor" ratio
Populations	5	7.08**	349**	.0007	27.4**	0.412**
Additive	1	29.95**	1533**	.0006	117.5**	1.634**
Dominance	1	0.12	25	.0006	4.1	0.092
Epistasis	3	1.77	62	.0008	5.2	0.112
Add-Add	1	3.55	140	.0005	12.6	0.189
Add-Dom	1	1.76	41	.0017	2.3	0.141
Dom-Dom	1	0.00	5	.0000	0.6	0.006
Error	614	0.75	42	.0005	3.3	0.081

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 72. Mean squares for the N fixation estimates/juvenile leaf width for Experiment VI.

Source	df	Acetylene reduced ratio	Shoot fresh weight ratio	Root fresh weight ratio	Shoot dry weight ratio	Nitrogen fixation "factor" ratio
Populations	5	143.0**	7017**	.0256	593**	9.07**
Additive	1	573.9**	24726**	.0005	1994**	25.38**
Dominance	1	24.1	6500*	.0740*	633**	12.61*
Epistasis	3	39.0	1286	.0178	113	2.45
Add-Add	1	75.6	2930	.0080	288	3.78
Add-Dom	1	41.3	885	.0437	48	3.50
Dom-Dom	1	.1	44	.0017	9	0.07
Error	614	21.3	1140	.0151	91	2.26

*significant at the 5% level of probability.
**significant at the 1% level of probability.

Table 73. Numbers of plants for shoot dry weight/cotyledon area ratio group by cotyledon area or juvenile leaf width crosstabulation for Experiment VI.

	Shoot dry weight/cotyledon area ratio			
	< 3.5	3.5-7.0	> 7.0	
Cotyledon area				Row total
< 32 mm^2	66	67	24	157
32-39 mm^2	74	52	4	130
39-42 mm^2	95	73	7	175
> 42 mm^2	82	76	4	162
Juvenile leaf width				Row total
< 6 mm	103	73	17	193
6-7 mm	133	103	16	252
> 7 mm	80	92	6	178
Column total	317	268	39	624

Table 74. Numbers of plants for shoot dry weight/juvenile leaf width ratio group by cotyledon area or juvenile leaf width crosstabulation for Experiment VI.

	Shoot dry weight/juvenile leaf width ratio			
	< 20	20-40	> 40	
Cotyledon area				Row total
< 32 mm^2	88	57	10	155
32-39 mm^2	88	37	4	129
39-42 mm^2	91	77	7	175
> 42 mm^2	74	83	5	162
Juvenile leaf width				Row total
< 6 mm	101	80	11	192
6-7 mm	143	98	11	252
> 7 mm	98	76	4	178
Column total	342	254	26	622

All analyses of the unadjusted estimates of nitrogen fixation revealed that all F_1 crosses exhibited heterosis when the plants were grown in a nitrogen limiting environment. However, when nitrogen is supplied in adequate amounts, there appears to be no difference between the F_1 or parental plants (McWhirter, 1969; Rotar and Chow, 1971). The heterosis in this study may be simply the expression of parental inbreeding depression when the plants are grown in a nitrogen limiting environment. These conclusions are supported by the negative estimates of the parental S.C.A. effects from the diallel studies (Table 50).

E. Breeding procedures for nitrogen fixation in Desmodium.

The analyses of this study revealed two major points concerning the inheritance of symbiotic nitrogen fixation in Desmodium. These hypotheses are:(a) that there was a major dominant gene conditioning strain-specific ineffective nodulation, and (b) that there was significant genetic variation for total symbiotic nitrogen fixation among effectively nodulated Desmodium genotypes and that this variation appears to have a heritable basis. Partitioning this genetic variance suggested that additive genetic variation and dominance variance were present with dominance variance being much larger when the estimates of nitrogen are not adjusted for juvenile plant vigor. Adjusting the estimates of nitrogen fixation for juvenile plant vigor revealed an almost totally additive (heritable) basis to the genetic variation.

1. Strain-specific ineffective nodulation genes.

The presence of strain-specific ineffective nodulation genes may be construed as a disadvantage when breeding for increased nitrogen fixation in *Desmodium*. However, these genes may also be used to advantage. If a *Desmodium* line or hybrid were to be released as a commercial cultivar it would be necessary to eliminate major genes causing ineffective nodulation. A rapid screening program with recurrent selection for the recessive genotype (effective nodulation) during early generation breeding trials would quickly remove ineffective nodulation genes from the potential cultivars.

The ineffective nodulation genes may also be used by a microbiologist as a means of selecting for nodulation competitiveness among strains of *Rhizobium* (Vincent, 1974). Having selected a series of *Desmodium* tester lines which carry at least one strain-specific ineffective nodulation gene, a microbiologist may initiate competition studies to rank the strains from most to least competitive for the nodulation sites. The most competitive strain if desirable in all other characteristics would be released as the commercial inoculum. The plant breeder having available a desirable strain of *Rhizobium* would begin to select or breed for a *Desmodium* hybrid or inbred line which would maximize the total fixation by the *Desmodium* line - commercial strain interaction.

2. Increasing total symbiotic nitrogen fixation.

The outstanding feature of the data from the quantitative experiments was the expression of heterosis. Those data clearly indicated that heterozygous genotypes of *Desmodium* were significantly superior to inbred lines for total symbiotic nitrogen fixation. A hybrid breeding program exploiting this heterosis would be desirable.

The reporting of cytoplasmic-genetic male-sterility in *Desmodium* (McWhirter, 1969) greatly assists the initiation of a hybrid breeding program. The male-sterile factor was found in line Ds1 and fertility was partially restored in crosses with line Da1. Male-sterile factors were also found in lines Ds2 and Da2. However fertility restoration was poor (McWhirter, pers. com.). Before initiating a hybrid breeding program for total fixation, complete fertility restoration would have to be established.

However, with the presence of significant average heterosis the accumulation of efficient inbred lines should be accompanied by a hybrid testing program. Such a breeding procedure has been referred to as reciprocal recurrent selection (Comstock *et al.*, 1949). Base populations for use in a reciprocal recurrent selection program could involve the phenotypically distinct yet fully fertile *D. sandwicense* by *D. aparines* or *D. aparines* by *D. uncinatum* crosses. Inbred lines necessary for this program could be developed either by mass selection, i.e. preservation of additive effects or by single seed descent (Goulden, 1939).

3. Nitrogen fixation components.

Heterosis or dominance genetic effects appear to be the rule when the nitrogen fixation estimates are analysed in their totality. However, when the total variation for each estimate is partitioned into components the dominance effects disappear. The genetic variation of each part is almost exclusively additive (heritable). In Experiment VI, two components of total nitrogen fixed were defined as; (a) juvenile plant vigor; estimated by cotyledon area and juvenile leaf width, and (b) "efficiency" of nitrogen fixation as estimated by the ratio of total nitrogen fixed to juvenile plant vigor. Large cotyledon and juvenile leaf size appeared to be conditioned by a dominant allele at a single locus. Plants with large cotyledons were shown to fix significantly more nitrogen so it would be advantageous at the early stages of a breeding program to select for large cotyledons or juvenile leaves. These plants would probably be more vigorous and therefore nodulate more quickly or form nodules at more sites. These results are similar to the results reported by Mytton (1973) where large seed weight assisted nodulation. It would be expected that selection for large cotyledons and juvenile leaves in Desmodium would rapidly respond to selection since a single gene appears to control these characters. Also achieved with this selection would be plants with increased seedling vigor which would establish a sward and begin fixing nitrogen and dry matter more quickly. Having selected a population of seedlings with large cotyledons or juvenile leaves the plants would enter the second phase of the breeding program; namely selection for "efficiency" of nitrogen fixation. Highly efficient plants would by tested against the selected Rhizobium strains (discussed in part 1 of this section) to determine the most favorable interaction which would be competitive for nodulation sites under field conditions. Such superior interactions could be selected for superior general adaptability or superior specific adaptability to a particular location which exhibits unique environmental conditions. If an interaction was selected for general adaptability this interaction would have to be tested under many locations, sites, and/or years since environmental conditions greatly effect total nitrogen fixation.

At this early stage in the development of the study of host variation for nitrogen fixation, the best avenue to pursue to achieve maximum return for research input is not clear. A hybrid breeding program working directly with heterosis may be more effective where capital and facilities are not limiting since the heritable variation is maintained under reciprocal recurrent selection. The limitation of the breeding program would be the numbers of individual plants which would have to be maintained to insure that to program was effective.

Initiating a breeding program emphasising the components of total nitrogen fixation would be more advantageous for laboratories with limiting resources. After selection for vigorous juvenile plants the populations could be effectively reduced by the elimination of the non-vigorous plants. The researcher could concentrate his resources to a smaller number of plants because the genetic basis of the components appears to be heritable and a selection response for greater "efficiency" would be expected.

It may be easy to speculate about the expected results of a breeding program employing either approach but the ultimate criterion judging the success of any research for increased nitrogen fixation would be a selection response culminating in a inbred variety or the creation of a superior hybrid which fixes a maximum amount of nitrogen under field conditions. Only when the products of research achieve practical results under agricultural conditions is the research program of merit or achieving its goals.

F. Relevance to tropical agriculture.

The importance of Desmodium in tropical agriculture is two-fold. Firstly, Desmodium being a forage legume would provide increased forage protein for animal consumption thereby effectively increasing availability of protein for human consumption. Secondly, Desmodium as a legume, fixes atmospheric nitrogen which assists non-leguminous plants to produce increased yield. If the total fixation could be increased Desmodium would be valuable in the tropics as a green manure crop and a soil conditioner as well as a forage legume.

The pending population increases in the tropics will dictate that the food chains be made as efficient as possible, i.e direct plant to human pathways for protein. D. sandwicense may be used in this context as a model organism for legume studies because of its many attributes for glasshouse growth. Principles of inheritance found in D. sandwicense may be directly applicable to other self-fertile grain legumes which are used for human consumption. The future of the need for adequate amounts of protein for human consumption cannot be underestimated and it is in this area where legumes may make a great contribution.

The secondary contribution of legumes would be as a rotational crop with cereals to buffer against catastrophies. In short, it would be optimal to develop annual grain legumes which fix more total nitrogen and increases protein production and rotate these legumes with the cereal crops which have been bred to produce maximum energy.

SUMMARY AND CONCLUSIONS

The genetic study of symbiotic nitrogen fixation in the tropical legume *Desmodium* was divided into three sections; namely, methodology, qualitative genetic variation, and quantitative genetic variation. The initial studies in the methodology section suggested that the bottle jar was effective for studying nitrogen fixation in *Desmodium*. Uninoculated or ineffectively nodulated control plants were yellow and chlorotic suggesting the lack of nitrogen. Green plants were taken from the bottle jars and analysed with the acetylene reduction assay. Dry weight accumulation was also recorded. Reduction of acetylene to ethylene preceeded at a linear rate for approximately 90 minutes. Linear reduction indicated that senescence of harvested *Desmodium* plants was not a limiting factor for the acetylene reduction analysis.

The within-bottle jar variation among similar genotypes suggested that the design of the bottle jar could be modified to reduce within-bottle jar variation. Modifications to the design and sand particle size greatly reduced this variation.

Initial nodulation studies indicated that some *D. aparines* lines were ineffectively nodulated by strain B. Studies of F_2 and backcross populations from *D. sandwicense* x *D. aparines* crosses implied that the strain-specific ineffective nodulation interaction between some *D. aparines* lines and strain B was conditioned by a dominant allele at a single locus.

The analysis of quantitative genetic variation suggested that several points were of importance. Firstly, the correlation between dry weight accumulation and the acetylene reduction assay was highly significant and there appeared to be a consistant pattern for both characters within an experiment. The acetylene reduction may be used as a plant breeding tool to screen juvenile Desmodium plants.

The most significant aspect illustrated by all quantitative experiments was the expression of F_1 heterosis. F_1 hybrids were significantly superior to inbred lines for total nitrogen fixed. A breeding program incorporating reciprocal recurrent selection would maximize the effect of F_1 heterosis. Although F_1 heterosis was significant, a portion of the genetic variance also appeared to be heritable and analysis of components of total nitrogen fixation predominantly expressed additive (heritable) genetic variation. Selecting inbred lines for these heritable effects would be beneficial since these lines could become the basis for recurrent selection programs or released as culivars.

Since genes controlling the formation of nitrogenase are found on the Rhizobium genome, quantitative variation in total nitrogen fixed may be due to physiological differences among host plants. The host plant may indirectly control symbiotic nitrogen fixation.

Genotype by environment interactions were of significant importance suggesting that studies concerning quantitative variation for symbiotic nitrogen fixation should be conducted under differing environments, locations, and/or years.

Breeding programs utilizing qualitative and quantitative genetic variation were outlined. Such a program would be useful for the production of highly efficient varieties. The relevance of such varieties to tropical agriculture was also discussed.

The preceding analyses suggested that the null hypothesis is to be rejected. The null hypothesis was that there was no genetic variation in the processes of the symbiotic relationship between *Desmodium* and *Rhizobium*. Two hypotheses may be postulated. These are: (a) that strain-specific ineffective nodulation interaction between strain B and some *D. aparines* lines was contitioned by a dominant allele at a single locus, and (b) that there was genetic variation in symbiotic nitrogen fixation in *Desmodium*.

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APPENDICIES

Appendix A: Crossing results from Colombia, S.A.

Table 1. Hybridization and emasculation observations using various washing solutions and flower preparations.

Treatment		Number of flowers manipulated	Percent pod set	Av. no. seeds per pod
Distilled water	- partial flower+	53	50	1.7
	- entire flower	32	84	3.9
	- emasculated	45	0	
2.5% sugar solution	- partial flower	28	60	3.4
	- entire flower	45	95	5.8
	- emasculated	63	5	2.3
5.0% sugar solution	- partial flower	43	46	1.8
	- emasculated	18	0	

+the tip half of the petals removed.

Table 2. The percent pod set and the average number of seeds per pod obtained during a 5x5 diallel cross.

Method	Number of flowers manipulated	Percent pod set	Av. no. seeds per pod	Number of seeds tested	Percent hybrids
Pollinated	387	8.2	4.3	104	95
Emasculated	60	6.6	2.5		

Table 3. The correlations between environmental factors and hybridization results.

	Percent pod set	Av. no. of seeds
Max. daily temp.	0.15	-.39
Min. daily temp.	0.30	-.19
Mean daily temp.	0.24	-.41
Temp. at pollination	0.07	-.17
SQRT(Daily precipitation)	-.24	0.19

SQRT = square root

Appendix B. Diallel analysis computer program listing.

1: DIALL subroutine instructions. 187

2: DIALL subroutine listing. 191

3: DIALL sample output. 206

Acknowledgment: A model I, method 1 diallel program was obtained from Mr. P. Hsu, Dept. of Plant Science, The Univ. of Alberta. This program was extensively edited, expanded and corrected by the author to produce an all model and method diallel program.

Subroutine name: DIALL

Subroutine title: Diallel analysis

Programmer: B. Pinchbeck

Program Description

Source Language: Fortran IV

Purpose: The analysis of a diallel cross for models I, II, and A; methods 1 to 4.

Description: DIALL is a algorithm which computes the diallel analysis of variance, components of genetic variance, Griffing's combining ability effects, or Gardner-Eberhart's constants. The subroutine generally follows the diallel analysis as outlined by Griffing (1956) yet is modified to include the analysis of variance of Hayman (1954) and Gardner and Eberhart (1966) for methods 1 and 2, respectively. The diallel table (p X p) or within-block diallel tables (p X p X b) are calculated from the formulae:

$$X_{ij} = \frac{1}{\mathrm{SQRT(bs)}} \Sigma\ Z_{ijkl}$$ (for a two-way randomized block design with interactions)

or

$$X_{ij} = \frac{1}{\mathrm{SQRT(b)}} \Sigma\ Z_{ijk}$$ (for a two-way randomized block design)

and

$$(X_{ij})_k = \frac{1}{\mathrm{SQRT(s)}} \Sigma\ (Z_{ij})_k \ ;\ k=1,b$$

and

$$S_{bg} = \Sigma_k (S_g)_k - S_g$$

where

p = number of diallel parents
b = number of blocks (replications, locations, years, etc.)
s = number of subsamples per cell
X = diallel table entry
Z = individual cell entries

X_{ij} = the ijth entry in the overall diallel table

$(X_{ij})_k$ = the ijth entry in the diallel table of the kth block.

S_{bg} = the sum of squares of the block x genetic component interaction

$(S_g)_k$ = the sum of squares for the genetic component in the kth block.

S_g = the sum of squares for the genetic component for the overall diallel table.

The above adjustments were incorporated so that the sum of the sum of squares of the main effect genetic components would equal to the treatment sum of squares for the two-way analysis of variance.

Griffing's combining ability effects and Gardner-Eberhart's constants are calculated from a diallel table of genotype means as outlined by Griffing (1956), and Gardner and Eberhart (1966), respectively.

Components of genetic variance are calculated from the diallel analysis of variance such that:

$\hat{V}_A = 2\hat{V}_g$ (inbred parents)

$\hat{V}_A = 4\hat{V}_g$ (noninbred parents)

$\hat{V}_D = \hat{V}_h + \hat{V}_r$ (methods 1 and 3; inbred parents)

$\hat{V}_D = 4\hat{V}_h + \hat{V}_r$ (methods 1 and 3; non-inbred parents)

$\hat{V}_D = \hat{V}_h$ (methods 2 and 4; inbred parents)

$\hat{V}_D = 4\hat{V}_h$ (methods 2 and 4; non-inbred parents)

$\hat{V}_G = \hat{V}_A + \hat{V}_D$

$\hat{V}_P = \hat{V}_G + \hat{V}_E$ (model A)

$\hat{V}_P = \hat{V}_G + \hat{V}_{BG} + \hat{V}_E$ (model II)

and where the estimated variance of the components are adjusted for block and subsamples. (for example)

$\hat{V}_g = \frac{1}{bs(p+2)} (M_g - M_h)$ (for model II, method 2)

whereas Griffing (1956) presents the formula:

$$\hat{V}_g = \frac{1}{p+2} (M_g - M_h)$$

and where

$\hat{V}_g$ = the general (G.C.A.) variance component

$\hat{V}_h$ = the heterosis (S.C.A.) variance component

$\hat{V}_r$ = the reciprocal variance component

$\hat{V}_A$ = the additive genetic variance component

$\hat{V}_D$ = the dominance genetic variance component

$\hat{V}_G$ = the genetic variance component

$\hat{V}_P$ = the phenotypic variance component

$\hat{V}_{BG}$ = the block x genotype variance component

$\hat{V}_E$ = the environmental variance component (error mean square)

Restrictions:

The subroutine is limited to an input diallel table matrix of genotype means of 10 parents x 10 parents x 10 blocks.

Program Usage

Calling Sequence: CALL DIALL (NP, NQ, NR, NS, MO, ME, NA, CH, IE, EMS, A, IW, IP)

Parameters:

Name	Purpose
NP -	number of parents
NQ -	rank of the third dimension of the diallel table
NR -	number of blocks in the original design
NS -	the number of subsamples per cell in the original design
MO -	diallel model number 0 - fixed model I - Gardner-Eberhart constants 1 - fixed model I - Griffing combining ability effects 2 - random model II - components of variation 3 - mixed model A - components of variation
ME -	diallel method number 1 = parents, F_1's and recipiocal F_1's

2 = parents and F_1's
3 = F_1's and recipiocal F_1's
4 = F_1's
0 = parents and F_1's (Gardner-Eberhart Analysis III)

NA - a vector describing the experiment (20A4)

CH - a vector describing the analysed character (10A4)

IE - error degrees of freedom

EMS- error mean square

A - matrix of block sums if NP x NP diallel table or subsample means if NP x NP x NQ diallel table

IW - logical I/O unit for output statements WRITE (IW, FMT)...

IP - 0 - if parents are inbred
1 - if parents are not inbred

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```
      SUBROUTINE DIALL(NP,NQ,NR,NS,MO,ME,NA,CH,IE,EMS,A,IW,IP)
C  DIALLEL ANALYSIS SUBROUTINE
C  VERSION 1.0, JUNE 1979
C
C  NP = NUMBER OF PARENTS  (MAX. 10)
C  NQ = NUMBER OF DIMENSIONS OF DIALLEL TABLE (MAX. 10)
C        (I.E. THE RANK OF THE THIRD DIMENSION OF 'A')
C  NR = NUMBER OF REPLICATIONS (BLOCKS,LOCATIONS,YEARS)
C       IN THE ORIGINAL DESIGN
C  NS = NUMBER OF SUBSAMPLES PER CELL IN THE ORIGINAL DESIGN
C  MO = DIALLEL MODEL NUMBER
C        0 = FIXED MODEL I - GARDNER-EBERHART CONSTANTS
C        1 = FIXED MODEL I - GRIFFING'S COMBINING ABILITY EFFECTS
C        2 = RANDOM MODEL II - COMPONENTS OF VARIANCE
C        3 = MIXED MODEL A - COMPONENTS OF VARIANCE
C  ME = DIALLEL METHOD NUMBER
C        0 = PARENTS AND F1'S (GARDNER-EBERHART ANALYSIS III)
C        1 = PARENTS,F1'S,AND RECIPROCAL F1'S
C        2 = PARENTS AND F1'S
C        3 = F1'S AND RECIPROCAL F1'S
C        4 = F1'S
C  NA = VECTOR DESCRIBING THE EXPERIMENT (20A4)
C  CH = VECTOR DESCRIBING THE CHARACTER BEING ANALYSED (10A4)
C  IE = ERROR DEGREES OF FREEDOM
C  EMS = ERROR MEAN SQUARE
C  A   = MATRIX OF BLOCK SUMS IF NP X NP DIALLEL TABLE OR
C       SUBSAMPLE MEANS IF NP X NP X NQ DIALLEL TABLE
C  IW = LOGICAL I/O UNIT FOR OUTPUT STATEMENTS : WRITE(IW,FMT)...
C  IP = 0 IF PARENTS ARE INBRED
C       1 IF PARENTS ARE NOT INBRED
C
C  PROGRAMMED BY BRIAN R. PINCHBECK
C
      DIMENSION A(10,10,10),NA(20),CH(10)
      DIMENSION S(10,10),R(10,10),X(10),Y(10),G(10),VS(10),VG(10)
      DOUBLE PRECISION RN,SN,A,S,R,X,Y,W,G,VS,VG,BT,BA,BD,BDD,BPD,BCD,BR
     &,BMR,BOR,SST,SSA,SSD,SSDD,SSPD,SSCD,SSR,SSMR,SSOR,TOT
      COMMON W(10,10)
      IA=NP-1
      ID=NP*IA/2
      ICD=NP*(NP-3)/2
      IOR=(NP-1)*(NP-2)/2
      IF (NS.LE.0) NS=1
      IF (ME.EQ.0) ID=ID-1
      PP=2.0
      PU=1.0
      PPP=1.0
      IF (IP.EQ.1) PP=4.0
      IF (IP.EQ.1) PPP=16.0
      IF (IP.EQ.1) PU=4.0
      IB=NR-1
      IBA=IB*IA
      IBD=IB*ID
      IBCD=IB*ICD
      IBOR=IB*IOR
      AI=IA
      DI=ID
      CDI=ICD
      ORI=IOR
      BI=IB
      BAI=IBA
      BDI=IBD
      BCDI=IBCD
      BORI=IBOR
      SN=NS
      RN=NR
      PN=NP
      BT=0.0
      BA=0.0
      BD=0.0
      BDD=0.0
      BPD=0.0
      BCD=0.0
      BR=0.0
      BMR=0.0
      BOR=0.0
      BMT=0.0
      BTI=0.0
      TOT=0.0
      ESS=EMS*IE
      LME=ME+1
      GO TO (190,10,190,360,550),LME
   10 CONTINUE
C  METHOD 1 ANALYSIS  OF VARIANCE
      IT=NP**2-1
      TI=IT
      IF (NQ.LE.1) GO TO 40
      IBT=IT*IB
      BTI=IBT
      DO 30 K=1,NQ
      DO 20 I=1,NP
      DO 20 J=1,NP
      W(I,J)=A(I,J,K)*DSQRT(SN)
   20 CONTINUE
      CALL DAV1(NP,SST,SSA,SSD,SSDD,SSPD,SSCD,SSR,SSMR,SSOR)
      BT=BT+SST
      BA=BA+SSA
      BD=BD+SSD
      BDD=BDD+SSDD
      BPD=BPD+SSPD
      BCD=BCD+SSCD
      BR=BR+SSR
      BMR=BMR+SSMR
      BOR=BOR+SSOR
   30 CONTINUE
   40 CONTINUE
      DO 50 I=1,NP
      DO 50 J=1,NP
      W(I,J)=0.0
      DO 50 K=1,NQ
      W(I,J)=W(I,J)+A(I,J,K)*DSQRT(SN)/DSQRT(RN)
   50 CONTINUE
      CALL DAV1(NP,SST,SSA,SSD,SSDD,SSPD,SSCD,SSR,SSMR,SSOR)
      BT=BT-SST
      BA=BA-SSA
      BD=BD-SSD
      BDD=BDD-SSDD
      BPD=BPD-SSPD
      BCD=BCD-SSCD
      BR=BR-SSR
      BMR=BMR-SSMR
      BOR=BOR-SSOR
      SMT=SST/TI
      SMA=SSA/AI
      SMD=SSD/DI
      SMPD=SSPD/AI
      SMCD=SSCD/CDI
      SMR=SSR/DI
      SMMR=SSMR/AI
      SMOR=SSOR/ORI
      FAT=SMT/EMS
      FBT=SMT/BMT
      FAA=SMA/EMS
      FAD=SMD/EMS
      FADD=SSDD/EMS
      FAPD=SMPD/EMS
      FACD=SMCD/EMS
      FAR=SMR/EMS
      FAMR=SMMR/EMS
      FAOR=SMOR/EMS
      PAT=FPROB(IT,IE,FAT)
      PAA=FPROB(IA,IE,FAA)
      PAD=FPROB(ID,IE,FAD)
      PADD=FPROB(1,IE,SSDD)
      PAPD=FPROB(IA,IE,FAPD)
      PACD=FPROB(ICD,IE,FACD)
      PAR=FPROB(ID,IE,FAR)
      PAMR=FPROB(IA,IE,FAMR)
      PAOR=FPROB(IOR,IE,FAOR)
      IF (NQ.LE.1) GO TO 60
      BMT=BT/BTI
      BMA=BA/BAI
      BMD=BD/BDI
      BMDD=BDD/BI
      BMPD=BPD/BAI
      BMCD=BCD/BCDI
      BRM=BR/BDI
      BMRM=BMR/BAI
      BORM=BOR/BORI
      FABT=BMT/EMS
      FABA=BMA/EMS
      FABD=BMD/EMS
      FABDD=BMDD/EMS
      FABPD=BMPD/EMS
      FABCD=BMCD/EMS
      FABR=BRM/EMS
      FABMR=BMRM/EMS
      FABOR=BORM/EMS
      PABT=FPROB(IBT,IE,FABT)
      PABA=FPROB(IBA,IE,FABA)
      PABD=FPROB(IBD,IE,FABD)
      PABDD=FPROB(IB,IE,FABDD)
      PABPD=FPROB(IBA,IE,FABPD)
      PABCD=FPROB(IBCD,IE,FABCD)
      PABR=FPROB(IBD,IE,FABR)
      PABMR=FPROB(IBA,IE,FABMR)
      PABOR=FPROB(IBCR,IE,FABOR)
   60 CONTINUE
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,720)
      WRITE (IW,740)
      WRITE (IW,1020) IT,SST,SMT,FAT,PAT
      WRITE (IW,750) IA,SSA,SMA,FAA,PAA
      WRITE (IW,760) ID,SSD,SMD,FAD,PAD
      WRITE (IW,770) SSDD,SSDD,FADD,PADD
      WRITE (IW,780) IA,SSPD,SMPD,FAPD,PAPD
      WRITE (IW,790) ICD,SSCD,SMCD,FACD,PACD
      WRITE (IW,800) ID,SSR,SMR,FAR,PAR
      WRITE (IW,810) IA,SSMR,SMMR,FAMR,PAMR
      WRITE (IW,820) IOR,SSOR,SMOR,FAOR,PAOR
      IF (NQ.LE.1) GO TO 70
      WRITE (IW,830) IBT,BT,BMT,FABT,PABT
      WRITE (IW,840) IBA,BA,BMA,FABA,PABA
      WRITE (IW,850) IBD,BD,BMD,FABD,PABD
      WRITE (IW,860) IB,BDD,BMDD,FABDD,PABDD
      WRITE (IW,870) IBA,BPD,BMPD,FABPD,PABPD
      WRITE (IW,880) IBCD,BCD,BMCD,FABCD,PABCD
      WRITE (IW,890) IBD,BR,BRM,FABR,PABR
      WRITE (IW,900) IBA,BMR,BMRM,FABMR,PABMR
      WRITE (IW,910) IBOR,BOR,BORM,FABOR,PABOR
   70 CONTINUE
      WRITE (IW,920) IE,ESS,EMS
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,1160) (I,I=1,NP)
      DO 90 I=1,NP
      X(I)=0.0
      Y(I)=0.0
      DO 80 J=1,NP
      W(I,J)=W(I,J)/DSQRT(SN)/DSQRT(RN)
      X(I)=X(I)+W(I,J)
      Y(I)=Y(I)+W(J,I)
      TOT=TOT+W(I,J)
   80 CONTINUE
      WRITE (IW,1080) I,(W(I,J),J=1,NP)
   90 CONTINUE
      XMU=TOT/PN**2
      SE1=SQRT(EMS/PN**2)
      WRITE (IW,1130) XMU,SE1
C  METHOD 1 COMPONENTS OF VARIANCE
      IF (MO.LT.2) GO TO 100
      WRITE (IW,730) ME,NA,CH
      CALL COV(NP,NR,NS,MO,SMA,SMD,SMR,BMT,EMS,AI,DI,BTI,IE,PP,PU,PPP)
      RETURN
  100 CONTINUE
      IF (MO.NE.1) GO TO 170
C  METHOD 1 GRIFFING'S COMBINING ABILITY ANALYSIS
      EMS=EMS/RN/SN
      DO 120 I=1,NP
      R(I,I)=0.0
      VS(I)=0.
      G(I)=((X(I)+Y(I))/(2.*PN))-XMU
      DO 120 J=I,NP
      S(I,J)=((W(I,J)+W(J,I))/2.)-((X(I)+X(J)+Y(I)+Y(J))/(2.*PN))+XMU
      S(J,I)=S(I,J)
      IF (I-J) 110,120,110
  110 CONTINUE
      VS(I)=VS(I)+S(I,J)**2
      R(I,J)=(W(I,J)-W(J,I))/2.
      R(J,I)=-R(I,J)
  120 CONTINUE
```

```
      DO 130 I=1,NP
      VG(I)=G(I)*G(I)-(PN-1.)/PN/PN/2.*EMS
      VS(I)=VS(I)/PN/2.-(PN-1.)/PN/2.*EMS
  130 CONTINUE
      SE2=SQRT((EMS*(PN-1.))/2./PN/PN)
      SE3=SQRT(EMS/PN)
      SE4=SQRT((EMS*(PN-1.)*(PN-1.))/PN/PN)
      SE5=SQRT((EMS*(PN*PN-2.*PN+2.))/2./PN/PN)
      SE6=SQRT(EMS*2.*(PN-2.)/PN)
      SE7=SQRT(EMS*(PN-1.)/PN)
      SE8=SQRT(EMS*(PN-2.)/PN)
      SE9=SQRT(EMS*(3.*PN-2.)/2./PN)
      SE10=SQRT(EMS*1.5*(PN-2.)/PN)
      SE11=SQRT(EMS/2.)
      SE12=SQRT(EMS)
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,1030)
      WRITE (IW,1040)
      DO 140 I=1,NP
      WRITE (IW,1050) I,G(I),VG(I),VS(I)
  140 CONTINUE
      WRITE (IW,1060) SE2,SE3
      WRITE (IW,1070) (I,I=1,NP)
      DO 150 I=1,NP
      WRITE (IW,1080) I,(S(I,J),J=1,I)
  150 CONTINUE
      WRITE (IW,1090) SE4,SE5,SE6,SE7,SE8
      WRITE (IW,1100) SE9,SE10
      WRITE (IW,1110) (I,I=1,NP)
      DO 160 I=1,NP
      WRITE (IW,1080) I,(R(I,J),J=1,NP)
  160 CONTINUE
      WRITE (IW,1120) SE11,SE12
      RETURN
  170 CONTINUE
      DO 180 I=1,NP
      DO 180 J=I,NP
      W(I,J)=(W(I,J)+W(J,I))/2.0
      W(J,I)=W(I,J)
  180 CONTINUE
C   GARDNER-EBERHART CONSTANTS
      WRITE (IW,730) ME,NA,CH
      CALL GARDCO(NP,ME,IW)
      RETURN
  190 CONTINUE
C   METHOD 2 ANALYSIS  OF VARIANCE
      IT=NP*(NP+1)/2-1
      TI=IT
      IF (NQ.LE.1) GO TO 220
      IBT=IT*IB
      BTI=IBT
      DO 210 K=1,NQ
      DO 200 I=1,NP
      DO 200 J=I,NP
      W(I,J)=A(I,J,K)*DSQRT(SN)
      W(J,I)=W(I,J)
  200 CONTINUE
      CALL DAV2(NP,ME,SST,SSA,SSD,SSDD,SSPD,SSCD)
      BT=BT+SST
      BA=BA+SSA
      BD=BD+SSD
      BDD=BDD+SSDD
      BPD=BPD+SSPD
      BCD=BCD+SSCD
  210 CONTINUE
  220 CONTINUE
      DO 230 I=1,NP
      DO 230 J=I,NP
      W(I,J)=0.0
      DO 230 K=1,NQ
      W(I,J)=W(I,J)+A(I,J,K)*DSQRT(SN)/DSQRT(RN)
      W(J,I)=W(I,J)
  230 CONTINUE
      CALL DAV2(NP,ME,SST,SSA,SSD,SSDD,SSPD,SSCD)
      BT=BT-SST
      BD=BD-SSD
      BA=BA-SSA
      BDD=BDD-SSDD
      BPD=BPD-SSPD
      BCD=BCD-SSCD
      SMT=SST/TI
      SMA=SSA/AI
      SMD=SSD/DI
      SMPD=SSPD/AI
      SMCD=SSCD/CDI
      FAT=SMT/EMS
      FAA=SMA/EMS
      FAD=SMD/EMS
      FADD=SSDD/EMS
      FAPD=SMPD/EMS
      FACD=SMCD/EMS
      PAT=FPROB(IT,IE,FAT)
      PAA=FPROB(IA,IE,FAA)
      PAD=FPROB(ID,IE,FAD)
      PADD=FPROB(1,IE,FADD)
      PAPD=FPROB(IA,IE,FAPD)
      PACD=FPROB(ICD,IE,FACD)
      IF (NQ.LE.1) GO TO 240
      BMT=BT/BTI
      BMA=BA/BAI
      BMD=BD/BDI
      BMDD=BDD/BI
      BMPD=BPD/BAI
      BMCD=BCD/BCDI
      FABT=BMT/EMS
      FABA=BMA/EMS
      FABD=BMD/EMS
      FABDD=BMDD/EMS
      FABPD=BMPD/EMS
      FABCD=BMCD/EMS
      PABT=FPROB(IBT,IE,FABT)
      PABA=FPROB(IBA,IE,FABA)
      PABD=FPROB(IBD,IE,FABD)
      PABDD=FPROB(IB,IE,FABDD)
      PABPD=FPROB(IBA,IE,FABPD)
      PABCD=FPROB(IBCD,IE,FABCD)
  240 CONTINUE
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,720)
      WRITE (IW,740)
      WRITE (IW,1020) IT,SST,SMT,FAT,PAT
      IF (ME.EQ.2) GO TO 250
      WRITE (IW,950) IA,SSPD,SMPD,FAPD,PAPD
      WRITE (IW,940) SSDD.SSDD,FADD,PADD
      WRITE (IW,930) ID,SSD,SMD,FAD,PAD
      WRITE (IW,960) IA.SSA,SMA,FAA,PAA
      WRITE (IW,980) ICD,SSCD,SMCD,FACD,PACD
      IF (NQ.LE.1) GO TO 260
      WRITE (IW,830) IBT,BT,BMT,FABT,PABT
      WRITE (IW,1010) IBA,BPD,BMPD,FABPD,PABPD
      WRITE (IW,1000) IB,BDD,BMDD,FABDD,PABDD
      WRITE (IW,990) IBD,BD,BMD.FABD,PABD
      WRITE (IW,970) IBA,BA,BMA,FABA,PABA
      WRITE (IW,880) IBCD,BCD,BMCD,FABCD,PABCD
      GO TO 260
  250 WRITE (IW,750) IA,SSA,SMA,FAA,PAA
      WRITE (IW,760) ID,SSD,SMD,FAD,PAD
      WRITE (IW,770) SSDD,SSDD,FADD,PADD
      WRITE (IW,780) IA,SSPD,SMPD,FAPD,PAPD
      WRITE (IW,790) ICD,SSCD,SMCD,FACD,PACD
      IF (NQ.LE.1) GO TO 260
      WRITE (IW,830) IBT,BT,BMT,FABT,PABT
      WRITE (IW,840) IBA,BA,BMA,FABA,PABA
      WRITE (IW,850) IBD,BD,BMD,FABD,PABD
      WRITE (IW,860) IB,BDD,BMDD,FABDD,PABDD
      WRITE (IW,870) IBA,BPD,BMPD,FABPD,PABPD
      WRITE (IW,880) IBCD,BCD,BMCD,FABCD,PABCD
  260 CONTINUE
      WRITE (IW,920) IE,ESS,EMS
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,1170) (I,I=1,NP)
      DO 290 I=1,NP
      X(I)=0.0
      DO 270 J=1,NP
      W(I,J)=W(I,J)/DSQRT(SN)/DSQRT(RN)
      X(I)=X(I)+W(I,J)
  270 CONTINUE
      DO 280 J=1,I
      TOT=TOT+W(I,J)
  280 CONTINUE
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      WRITE (IW,1080) I,(W(I,J),J=1,I)
  290 CONTINUE
      XMU=TOT*2./PN/(PN+1.)
      SE1=SQRT(EMS*2./PN/(PN+1.))
      WRITE (IW,1130) XMU,SE1
C  METHOD 2 COMPONENTS OF VARIANCE
      IF (MO.LT.2) GO TO 300
      WRITE (IW,730) ME,NA,CH
      CALL COV(NP,NR,NS,MO,SMA,SMD,0.0,BMT,EMS,AI,DI,BTI,IE,PP,PU,PPP)
      RETURN
  300 CONTINUE
C  GARDNER-EBERHART CONSTANTS
      IF (MO.NE.0) GO TO 310
      WRITE (IW,730) ME,NA,CH
      CALL GARDCO(NP,ME,IW)
      RETURN
  310 CONTINUE
C  METHOD 2 GRIFFING'S COMBINING ABILITY ANALYSIS
      EMS=EMS/RN/SN
      DO 320 I=1,NP
      VS(I)=0.0
      G(I)=((X(I)+W(I,I))-(2.*TOT)/PN)/(PN+2.)
      DO 320 J=I,NP
      S(I,J)=W(I,J)-((X(I)+X(J)+W(I,I)+W(J,J))/(PN+2.))+((2.*TOT)/((PN+
     &1.)*(PN+2.)))
      S(J,I)=S(I,J)
      IF (I.EQ.J) GO TO 320
      VS(I)=VS(I)+S(I,J)**2
  320 CONTINUE
      DO 330 I=1,NP
      VG(I)=G(I)**2-(((PN-1.)/(PN*(PN-2.)))*EMS)
      VS(I)=(VS(I)/(PN-2.))-(((PN-3.)/(PN-2.))*EMS)
  330 CONTINUE
      SE2=SQRT(((PN-1.)/(PN*(PN+2.)))*EMS)
      SE3=SQRT((2.*EMS)/(PN+2.))
      SE4=SQRT(((PN*(PN-1.))/((PN+1.)*(PN+2.)))*EMS)
      SE5=SQRT(((PN**2+PN+2.)/((PN+1.)*(PN+2.)))*EMS)
      SE6=SQRT(((2.*(PN-2.))/(PN+2.))*EMS)
      SE7=SQRT(((2.*(PN+1.))/(PN+2.))*EMS)
      SE8=SQRT(((2.*PN)/(PN+2.))*EMS)
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,1030)
      WRITE (IW,1040)
      DO 340 I=1,NP
      WRITE (IW,1050) I,G(I),VG(I),VS(I)
  340 CONTINUE
      WRITE (IW,1060) SE2,SE3
      WRITE (IW,1070) (I,I=1,NP)
      DO 350 I=1,NP
      WRITE (IW,1080) I,(S(I,J),J=1,I)
  350 CONTINUE
      WRITE (IW,1090) SE4,SE5,SE6,SE7,SE8
      RETURN
  360 CONTINUE
C  METHOD 3 ANALYSIS OF VARIANCE
      IT=NP**2-NP-1
      TI=IT
      IF (NQ.LE.1) GO TO 390
      IBT=IT*IB
      BTI=IBT
      DO 380 K=1,NQ
      DO 370 I=1,NP
      DO 370 J=1,NP
      W(I,J)=A(I,J,K)*DSQRT(SN)
  370 CONTINUE
      CALL DAV3(NP,SST,SSA,SSD,SSR,SSMR,SSOR)
      BT=BT+SST
      BA=BA+SSA
      BD=BD+SSD
      BR=BR+SSR
      BMR=BMR+SSMR
      BOR=BOR+SSOR
  380 CONTINUE
  390 CONTINUE
      DO 400 I=1,NP
      DO 400 J=1,NP
      W(I,J)=0.0
      DO 400 K=1,NQ
      W(I,J)=W(I,J)+A(I,J,K)*DSQRT(SN)/DSQRT(RN)
  400 CONTINUE
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      CALL DAV3(NP,SST,SSA,SSD,SSR,SSMR,SSOR)
      BT=BT-SST
      BA=BA-SSA
      BD=BD-SSD
      BR=BR-SSR
      BMR=BMR-SSMR
      BOR=BOR-SSOR
      SMT=SST/TI
      SMA=SSA/AI
      SMD=SSD/CDI
      SMR=SSR/DI
      SMMR=SSMR/AI
      SMOR=SSOR/ORI
      FAT=SMT/EMS
      FAA=SMA/EMS
      FAD=SMD/EMS
      FAR=SMR/EMS
      FAMR=SMMR/EMS
      FAOR=SMOR/EMS
      PAT=FPROB(IT,IE,FAT)
      PAA=FPROB(IA,IE,FAA)
      PAD=FPROB(ID,IE,FAD)
      PAR=FPROB(ID,IE,FAR)
      PAMR=FPROB(IA,IE,FAMR)
      PAOR=FPROB(IOR,IE,FAOR)
      IF (NQ.LE.1) GO TO 410
      BMT=BT/BTI
      BMA=BA/BAI
      BMD=BD/BCDI
      BRM=BR/BDI
      BMMR=BMR/BAI
      BMOR=BOR/BORI
      FABT=BMT/EMS
      FABA=BMA/EMS
      FABD=BMD/EMS
      FABR=BRM/EMS
      FABMR=BMMR/EMS
      FABOR=BMOR/EMS
      PABT=FPROB(IBT,IE,FABT)
      PABA=FPROB(IBA,IE,FABA)
      PABD=FPROB(IBD,IE,FABD)
      PABR=FPROB(IBD,IE,FABR)
      PABMR=FPROB(IBA,IE,FABMR)
      PABOR=FPROB(IBCR,IE,FABOR)
  410 CONTINUE
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,720)
      WRITE (IW,740)
      WRITE (IW,1020) IT,SST,SMT,FAT,PAT
      WRITE (IW,750) IA,SSA,SMA,FAA,PAA
      WRITE (IW,760) ICD,SSD,SMD,FAD,PAD
      WRITE (IW,800) ID,SSR,SMR,FAR,PAR
      WRITE (IW,810) IA,SSMR,SMMR,FAMR,PAMR
      WRITE (IW,820) IOR,SSOR,SMOR,FAOR,PAOR
      IF (NQ.LE.1) GO TO 420
      WRITE (IW,830) IBT,BT,BMT,FABT,PABT
      WRITE (IW,840) IBA,BA,BMA,FABA,PABA
      WRITE (IW,850) IBCD,BD,BMD,FABD,PABD
      WRITE (IW,890) IBD,BR,BRM,FABR,PABR
      WRITE (IW,900) IBA,BMR,BMMR,FABMR,PABMR
      WRITE (IW,910) IBOR,BOR,BMOR,FABOR,PABOR
  420 CONTINUE
      WRITE (IW,920) IE,ESS,EMS
      EMS=EMS/RN/SN
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,1160) (I,I=1,NP)
      DO 440 I=1,NP
      W(I,I)=0.0
      X(I)=0.0
      Y(I)=0.0
      DO 430 J=1,NP
      W(I,J)=W(I,J)/DSQRT(SN)/DSQRT(RN)
      X(I)=X(I)+W(I,J)
      Y(I)=Y(I)+W(J,I)
      TOT=TOT+W(I,J)
  430 CONTINUE
      WRITE (IW,1080) I,(W(I,J),J=1,NP)
  440 CONTINUE
      XMU=TOT/PN/(PN-1.)
      SE1=SQRT(EMS/PN/(PN-1.))
      WRITE (IW,1130) XMU,SE1
C   METHOD 3 COMPONENTS OF VARIANCE
      IF (MO.LT.2) GO TO 450
      WRITE (IW,730) ME,NA,CH
      CALL COV(NP,NR,NS,MO,SMA,SMD,SMR,BMT,EMS,AI,DI,BTI,IE,PP,PU,PPP)
      RETURN
  450 CONTINUE
      IF (MO.NE.1) GO TO 530
C   METHOD 3 GRIFFING'S COMBINING ABILITY ANALYSIS
      DO 460 I=1,NP
      R(I,I)=0.0
      S(I,I)=0.0
      VS(I)=0.0
  460 CONTINUE
      IG=NP-1
      DO 470 I=1,IG
      K=I+1
      DO 470 J=K,NP
      S(I,J)=(W(I,J)+W(J,I))/2.-(X(I)+X(J)+Y(I)+Y(J))/2./(PN-2.)+TOT/(PN
     &-1.)/(PN-2.)
      R(I,J)=(W(I,J)-W(J,I))/2.
      S(J,I)=S(I,J)
      R(J,I)=-R(I,J)
  470 CONTINUE
      DO 490 I=1,NP
      DO 480 J=1,NP
      VS(I)=VS(I)+S(I,J)**2
  480 CONTINUE
      G(I)=(PN*(X(I)+Y(I))-2.*TOT)/2./PN/(PN-2.)
      VG(I)=G(I)**2-EMS*(PN-1.)/PN/(PN-2.)/2.
      VS(I)=VS(I)/(PN-2.)/2.-EMS*(PN-3.)/(PN-2.)/2.
  490 CONTINUE
      SE2=SQRT(EMS*(PN-1.)/2./PN/(PN-2.))
      SE3=SQRT(EMS/(PN-2.))
      SE4=SQRT(EMS*(PN-3.)/2./(PN-1.))
      SE5=SQRT(EMS*(PN-3.)/(PN-2.))
      SEL=SQRT(EMS*(PN-4.)/(PN-2.))
      SE7=SQRT(EMS/2.)
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,1030)
      WRITE (IW,1040)
      DO 500 I=1,NP
      WRITE (IW,1050) I,G(I),VG(I),VS(I)
  500 CONTINUE
      WRITE (IW,1060) SE2,SE3
      WRITE (IW,1070) (I,I=1,NP)
      DO 510 I=1,NP
      WRITE (IW,1080) I,(S(I,J),J=1,I)
  510 CONTINUE
      WRITE (IW,1140) SE4,SE5,SE6
      WRITE (IW,1110) (I,I=1,NP)
      DO 520 I=1,NP
      WRITE (IW,1080) I,(R(I,J),J=1,NP)
  520 CONTINUE
      WRITE (IW,1150) SE7
      RETURN
  530 CONTINUE
      DO 540 I=1,NP
      DO 540 J=I,NP
      W(I,J)=(W(I,J)+W(J,I))/2.0
      W(J,I)=W(I,J)
  540 CONTINUE
C   GARDNER-EBERHART CONSTANTS
      WRITE (IW,730) ME,NA,CH
      CALL GARDCO(NP,ME,IW)
      RETURN
C   METHOD 4 ANALYSIS OF VARIANCE
  550 CONTINUE
      IT=NP*(NP-1)/2-1
      TI=IT
      IF (NQ.LE.1) GO TO 580
      IBT=IT*IB
      BTI=IBT
      DO 570 K=1,NQ
      DO 560 I=1,NP
      DO 560 J=I,NP
      W(I,J)=A(I,J,K)*DSQRT(SN)
      W(J,I)=W(I,J)
  560 CONTINUE
      CALL DAV4(NP,SST,SSA,SSD)
      BT=BT+SST
      BA=BA+SSA
      BD=BD+SSD
  570 CONTINUE
  580 CONTINUE
      DO 590 I=1,NP
      DO 590 J=I,NP
      W(I,J)=0.0
      DO 590 K=1,NQ
      W(I,J)=W(I,J)+A(I,J,K)*DSQRT(SN)/DSQRT(RN)
      W(J,I)=W(I,J)
  590 CONTINUE
      CALL DAV4(NP,SST,SSA,SSD)
      BT=BT-SST
      BA=BA-SSA
      BD=BD-SSD
      SMT=SST/TI
      SMA=SSA/AI
      SMD=SSD/CDI
      FAT=SMT/EMS
      FAA=SMA/EMS
      FAD=SMD/EMS
      PAT=FPROB(IT,IE,FAT)
      PAA=FPROB(IA,IE,FAA)
      PAD=FPROB(ID,IE,FAD)
      IF (NQ.LE.1) GO TO 600
      BMT=BT/BTI
      BMA=BA/BAI
      BMD=BD/BCDI
      FABT=BMT/EMS
      FABA=BMA/EMS
      FABD=BMD/EMS
      PABT=FPROB(IBT,IE,FABT)
      PABA=FPROB(IBA,IE,FABA)
      PABD=FPROB(IBD,IE,FABD)
  600 CONTINUE
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,720)
      WRITE (IW,740)
      WRITE (IW,1020) IT,SST,SMT,FAT,PAT
      WRITE (IW,750) IA,SSA,SMA,FAA,PAA
      WRITE (IW,760) ICD,SSD,SMD,FAD,PAD
      IF (NQ.LE.1) GO TO 610
      WRITE (IW,830) IBT,BT,BMT,FABT,PABT
      WRITE (IW,840) IBA,BA,BMA,FABA,PABA
      WRITE (IW,850) IBCD,BD,BMD,FABD,PABD
  610 CONTINUE
      WRITE (IW,920) IE,ESS,EMS
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,1170) (I,I=1,NP)
      DO 640 I=1,NP
      W(I,I)=0.0
      X(I)=0.0
      DO 620 J=1,NP
      W(I,J)=W(I,J)/DSQRT(SN)/DSQRT(RN)
      X(I)=X(I)+W(I,J)
  620 CONTINUE
      DO 630 J=I,NP
      TOT=TOT+W(I,J)
  630 CONTINUE
      WRITE (IW,1080) I,(W(I,J),J=1,I)
  640 CONTINUE
      SE1=SQRT(EMS*2./PN/(PN-1.))
      XMU=2.*TOT/PN/(PN-1.)
      WRITE (IW,1130) XMU,SE1
C  METHOD 4 COMPONENTS OF VARIANCE
      IF (MO.LT.2) GO TO 650
      WRITE (IW,730) ME,NA,CH
      CALL COV(NP,NR,NS,MO,SMA,SMD,0.0,BMT,EMS,AI,DI,BTI,IE,PP,PU,PPP)
      RETURN
  650 CONTINUE
C  GARDNER-EBERHART CONSTANTS
      IF (MO.NE.0) GO TO 660
      WRITE (IW,730) ME,NA,CH
      CALL GARDCO(NP,ME,IW)
      RETURN
  660 CONTINUE
C  METHOD 4 GRIFFING'S COMBINING ABILITY ANALYSIS
      EMS=EMS/RN/SN
      DO 670 I=1,NP
      S(I,I)=0.0
      DO 670 J=I,NP
      IF (I.EQ.J) GO TO 670
      S(I,J)=W(I,J)-(X(I)+X(I))/(PN-2.)+2.*TOT/(PN-1.)/(PN-2.)
      S(J,I)=S(I,J)
  670 CONTINUE
      DO 690 I=1,NP
      DO 680 J=I,NP
      VS(I)=VS(I)+S(I,J)**2
  680 CONTINUE
      G(I)=(PN*X(I)-2.*TOT)/PN/(PN-2.)
      VG(I)=G(I)**2-EMS*(PN-1.)/PN/(PN-2.)
      VS(I)=VS(I)/(PN-2.)-EMS*(PN-3.)/(PN-2.)
  690 CONTINUE
      SE2=SQRT(EMS*(PN-1.)/PN/(PN-2.))
      SE3=SQRT(EMS*2./(PN-2.))
      SE4=SQRT(EMS*(PN-3.)/(PN-1.))
      SE5=SQRT(EMS*2.*(PN-3.)/(PN-2.))
      SE6=SQRT(EMS*2.*(PN-4.)/(PN-2.))
      WRITE (IW,730) ME,NA,CH
      WRITE (IW,1030)
      WRITE (IW,1040)
      DO 700 I=1,NP
      WRITE (IW,1050) I,G(I),VG(I),VS(I)
  700 CONTINUE
      WRITE (IW,1060) SE2,SE3
      WRITE (IW,1070) (I,I=1,NP)
      DO 710 I=1,NP
      WRITE (IW,1080) I,(S(I,J),J=1,I)
  710 CONTINUE
      WRITE (IW,1140) SE4,SE5,SE6
      RETURN
  720 FORMAT (' ','ANALYSIS OF VARIANCE'/)
  730 FORMAT ('1','METHOD',I2,' DIALLEL ANALYSIS'//1X,20A4//1X,10A4//)
  740 FORMAT ('0','SOURCE OF VARIATION',12X,'DF',8X,'SUM OF SQUARES',8X,
     &'MEAN SQUARES',18X,'F',14X,'P')
  750 FORMAT ('0',2X,'GENERAL (GCA)',15X,I3,2F20.4,10X,2F15.4)
  760 FORMAT ('0',2X,'HETEROSIS (SCA)',13X,I3,2F20.4,10X,2F15.4)
  770 FORMAT ('0',4X,'AVERAGE HETEROSIS',11X,'1',2F20.4,10X,2F15.4)
  780 FORMAT ('0',4X,'VARIETY HETEROSIS',9X,I3,2F20.4,10X,2F15.4)
  790 FORMAT ('0',4X,'SPECIFIC HETEROSIS',8X,I3,2F20.4,10X,2F15.4)
  800 FORMAT ('0',2X,'RECIPROCAL',18X,I3,2F20.4,10X,2F15.4)
  810 FORMAT ('0',4X,'MATERNAL RECIPROCAL',7X,I3,2F20.4,10X,2F15.4)
  820 FORMAT ('0',4X,'NON-MATERNAL RECIPROCAL',3X,I3,2F20.4,10X,2F15.4)
  830 FORMAT ('0','GENOTYPE INTERACTION',10X,I3,2F20.4,10X,2F15.4)
  840 FORMAT ('0',2X,'GENERAL INTERACTION',9X,I3,2F20.4,10X,2F15.4)
  850 FORMAT ('0',2X,'HETEROSIS INTERACTION',7X,I3,2F20.4,10X,2F15.4)
  860 FORMAT ('0',4X,'AVERAGE INTERACTION',7X,I3,2F20.4,10X,2F15.4)
  870 FORMAT ('0',4X,'VARIETY INTERACTION',7X,I3,2F20.4,10X,2F15.4)
  880 FORMAT ('0',4X,'SPECIFIC INTERACTION',6X,I3,2F20.4,10X,2F15.4)
  890 FORMAT ('0',2X,'RECIPROCAL INTERACTION',6X,I3,2F20.4,10X,2F15.4)
  900 FORMAT ('0',4X,'MATERNAL INTERACTION',6X,I3,2F20.4,10X,2F15.4)
  910 FORMAT ('0',4X,'NON-MATERNAL INTERACTION',2X,I3,2F20.4,10X,2F15.4)
  920 FORMAT ('0','ERROR',24X,I4,2F20.4//)
  930 FORMAT ('0',2X,'CROSSES',21X,I3,2F20.4,10X,2F15.4)
  940 FORMAT ('0',2X,'VARIETIES VS CROSSES',10X,'1',2F20.4,10X,2F15.4)
  950 FORMAT ('0',2X,'VARIETIES',19X,I3,2F20.4,10X,2F15.4)
  960 FORMAT ('0',4X,'GENERAL (GCA)',13X,I3,2F20.4,10X,2F15.4)
  970 FORMAT ('0',4X,'GENERAL INTERACTION',7X,I3,2F20.4,10X,2F15.4)
  980 FORMAT ('0',4X,'SPECIFIC (SCA)',12X,I3,2F20.4,10X,2F15.4)
  990 FORMAT ('0',2X,'CROSSES INTERACTION',9X,I3,2F20.4,10X,2F15.4)
 1000 FORMAT ('0',2X,'VARIETIES VS CROSSES INT.',3X,I3,2F20.4,10X,2F15.4
     &)
 1010 FORMAT ('0',2X,'VARIETIES INTERACTION',7X,I3,2F20.4,10X,2F15.4)
 1020 FORMAT ('0','GENOTYPES',21X,I3,2F20.4,10X,2F15.4)
 1030 FORMAT ('0','GRIFFING''S COMBINING ABILITY ANALYSIS (MODEL I)'/)
 1040 FORMAT ('0',10X,'PARENT',5X,'G.C.A. EFFECTS',7X,'G.C.A. VARIANCE',
     &7X,'S.C.A. VARIANCE'/)
 1050 FORMAT (' ',12X,I2,9X,F10.4,2(10X,F12.4))
 1060 FORMAT ('0',10X,'S.E. OF THE G.C.A. EFFECTS -',20X,F10.4/11X,'S.E.
     & OF THE DIFFERENCES BETWEEN G.C.A. EFFECTS -',F10.4//)
 1070 FORMAT ('0',10X,'S.C.A. EFFECTS'//6X,'PARENTS',4X,I5,9I10)
 1080 FORMAT ('0',11X,I2,2X,10F10.4)
 1090 FORMAT ('0',10X,'S.E. OF THE PARENTAL S.C.A. EFFECTS -',35X,F10.4/
     &11X,'S.E. OF THE CROSS S.C.A. EFFECTS -',38X,F10.4/11X,'S.E. OF TH
     &E PARENTAL DIFFERENCES (S.C.A. EFFECTS) -',21X,F10.4/11X,'S.E. OF
     &THE COMMON PARENT CROSS DIFFERENCES (S.C.A. EFFECTS) -',10X,F10.4/
     &11X,'S.E. OF THE DIFFERENT PARENT CROSS DIFFERENCES (S.C.A. EFFECT
     &S) -',7X,F10.4)
 1100 FORMAT (' ',10X,'S.E. OF THE PARENT MINUS PROGENY DIFFERENCES (S.C
     &.A. EFFECTS) -',9X,F10.4/11X,'S.E. OF THE PARENT MINUS NON-PROGENY
     & DIFFERENCES (S.C.A. EFFECTS) -',5X,F10.4)
 1110 FORMAT ('0',10X,'RECIPROCAL EFFECTS'//25X,' MALE PARENT'/' FEMALE
     &PARENT',I8,9I10)
 1120 FORMAT ('0',10X,'S.E. OF THE RECIPROCAL EFFECTS -',40X,F10.4/11X,'
     &S.E. OF THE DIFFERENCES BETWEEN RECIPROCAL EFFECTS -',20X,F10.4//)
 1130 FORMAT ('0','POPULATION MEAN - ',F10.4,5X,'S.E. - ',F10.4//)
 1140 FORMAT ('0',10X,'S.E. OF S.C.A. EFFECTS - ',48X,F10.4/11X,'S.E. OF
     & THE COMMON PARENT CROSS DIFFERENCES (S.C.A. EFFECTS) - ',10X,
     &F10.4/11X,'S.E. OF THE DIFFERENT PARENT CROSS DIFFERENCES (S.C.A.
     &EFFECTS) - ',7X,F10.4)
 1150 FORMAT ('0','S.E. OF THE RECIPROCAL EFFECTS - ',40X,F10.4)
 1160 FORMAT ('0','DIALLEL MEANS'//25X,'MALE PARENT'/' FEMALE PARENT',I8
     &,9I10)
 1170 FORMAT ('0','DIALLEL MEANS'//6X,'PARENTS',4X,I5,9I10)
      END
      SUBROUTINE DAV1(NP,SST,SSA,SSD,SSDD,SSPD,SSCD,SSR,SSMR,SSOR)
      DIMENSION X(10),Y(10)
      DOUBLE PRECISION PN,X,Y,W,SST,SSA,SSD,SSDD,SSPD,SSCD,SSR,SSMR,SSOR
     &,X1,X2,X4,X6,X8,H2,H4,H6,TO,SP,SSP
      COMMON W(10,10)
      PN=NP
      IA=NP-1
      X1=0.0
      X2=0.0
      X4=0.0
      X6=0.0
      X8=0.0
      H2=0.0
      H4=0.0
      H6=0.0
      TO=0.0
      SP=0.0
      SSP=0.0
      DO 10 I=1,NP
      X(I)=0.0
      Y(I)=0.0
      DO 10 J=1,NP
      X1=X1+W(I,J)**2
      X(I)=X(I)+W(J,I)
      Y(I)=Y(I)+W(I,J)
   10 CONTINUE
      DO 20 I=1,NP
      X2=X2+(X(I)+Y(I))**2
      X4=X4+(X(I)-Y(I))**2
      X6=X6+(X(I)+Y(I)-2.*W(I,I))**2
      X8=X8+(X(I)+Y(I)-PN*W(I,I))**2
      TO=TO+X(I)
      SP=SP+W(I,I)
      SSP=SSP+W(I,I)**2
      DO 20 J=1,NP
      H2=H2+(W(I,J)+W(J,I))**2
   20 CONTINUE
      DO 30 I=1,IA
      DO 30 K=I,IA
      J=K+1
      H4=H4+(W(I,J)-W(J,I))**2
      H6=H6+(W(I,J)+W(J,I))**2
   30 CONTINUE
      SST=X1-TO**2/PN/PN
      SSA=(X2/2./PN)-(2.*TO**2/PN/PN)
      SSD=(H2/4.)-(X2/2./PN)+(TO**2/PN/PN)
      SSDD=(TO-(PN*SP))**2/(PN-1.)/PN**2
      SSPD=(X8-(((2.*TO-PN*SP)**2)/PN))/PN/(PN-2.)
      SSCD=(H6/2.)-(X6/2./(PN-2.))+((TO-SP)**2/(PN-2.)/(PN-1.))
      SSR=H4/2.
      SSMR=X4/2./PN
      SSOR=SSR-SSMR
      RETURN
      END
      SUBROUTINE DAV2(NP,ME,SST,SSA,SSD,SSDD,SSPD,SSCD)
      DIMENSION X(10)
      DOUBLE PRECISION PN,X,W,SST,SSA,SSD,SSDD,SSPD,SSCD,SSAA,SP,TO,X2,
     &X4,X6,X8,H2,SSP
      COMMON W(10,10)
      PN=NP
      SP=0.0
      TO=0.0
      X2=0.0
      X4=0.0
      X6=0.0
      X8=0.0
      H2=0.0
      SSP=0.0
      DO 20 I=1,NP
      X(I)=0.0
      SP=SP+W(I,I)
      SSP=SSP+W(I,I)**2
      DO 10 J=1,NP
      X(I)=X(I)+W(I,J)
   10 CONTINUE
      X2=X2+(X(I)+W(I,I))**2
      X4=X4+(X(I)-W(I,I))**2
      X6=X6+(X(I)+W(I,I)-((PN+2.)/2.)*W(I,I))**2
      DO 20 J=I,NP
      H2=H2+W(I,J)**2
      TO=TO+W(I,J)
   20 CONTINUE
      SST=H2-2.*TO**2/PN/(PN+1.)
      SSA=(X2-4.*TO**2/PN)/(PN+2.)
      SSAA=X4/(PN-2.)-4.*(TO-SP)**2/PN/(PN-2.)
      SSD=H2-X2/(PN+2.)+2.*TO**2/(PN+1.)/(PN+2.)
      SSDD=(TO-(PN+1.)/2.*SP)**2*4./PN/(PN**2-1.)
      SSPD=4.*(X6-((2.*TO-(PN+2.)/2.*SP)**2/PN))/(PN**2-4.)
      SSCD=(H2-SSP)-X4/(PN-2.)+2.*(TO-SP)**2/(PN-1.)/(PN-2.)
      IF (ME.NE.0) RETURN
      SSPD=SSPD+SSA-SSAA
      SSA=SSAA
      SSD=SSA+SSCD
      RETURN
      END
      SUBROUTINE DAV3(NP,SST,SSA,SSD,SSR,SSMR,SSOR)
      DIMENSION X(10),Y(10)
      DOUBLE PRECISION PN,X,Y,W,TO,SST,SSA,SSD,SSR,SSMR,SSOR,X1,X2,X4,H2
     &,H4
      COMMON W(10,10)
      PN=NP
      TO=0.0
      X1=0.0
      X2=0.0
      X4=0.0
      H2=0.0
      H4=0.0
      DO 20 I=1,NP
      W(I,I)=0.0
      X(I)=0.0
      Y(I)=0.0
      DO 10 J=1,NP
      X1=X1+W(I,J)**2
      X(I)=X(I)+W(J,I)
      Y(I)=Y(I)+W(I,J)
   10 CONTINUE
      TO=TO+X(I)
      X2=X2+(X(I)+Y(I))**2
      X4=X4+(X(I)-Y(I))**2
      DO 20 J=I,NP
      H2=H2+(W(I,J)+W(J,I))**2
      H4=H4+(W(I,J)-W(J,I))**2
   20 CONTINUE
      SST=X1-TO**2/PN/(PN-1.)
      SSA=X2/2./(PN-2.)-2.*TO**2/PN/(PN-2.)
      SSD=H2/2.-X2/2./(PN-2.)+TO**2/(PN-1.)/(PN-2.)
      SSR=H4/2.
      SSMR=X4/2./PN
      SSOR=SSR-SSMR
      RETURN
      END
      SUBROUTINE DAV4(NP,SST,SSA,SSD)
      DIMENSION X(10)
      DOUBLE PRECISION PN,X,W,SST,SSA,SSD,X2,H2,TO
      COMMON W(10,10)
      PN=NP
      X2=0.0
      H2=0.0
      TO=0.0
      DO 20 I=1,NP
      W(I,I)=0.0
      X(I)=0.0
      DO 10 J=1,NP
      X(I)=X(I)+W(I,J)
   10 CONTINUE
      X2=X2+X(I)**2
      DO 20 J=I,NP
      H2=H2+W(I,J)**2
      TO=TO+W(I,J)
   20 CONTINUE
      SST=H2-2.*TO**2/PN/(PN-1.)
      SSA=X2/(PN-2.)-4.*TO**2/PN/(PN-2.)
      SSD=H2-X2/(PN-2.)+2.*TO**2/(PN-1.)/(PN-2.)
      RETURN
      END
      SUBROUTINE GARDCO(NP,ME,IW)
C   GARDNER-EBERHART DIALLEL CONSTANTS SUBROUTINE
C   NP = NUMBER OF PARENTS
C   ME = DIALLEL METHOD NUMBER
C          0 = PARENTS AND F1'S (GARDNER-EBERHART ANALYSIS III)
C          1 = PARENTS,F1'S,AND RECIPROCAL F1'S
C          2 = PARENTS AND F1'S
C          3 = F1'S AND RECIPROCAL F1'S
C          4 = F1'S
C   IW = LOGICAL I/O UNIT FOR OUTPUT STATEMENTS : WRITE(IW,FMT)...
C    W = (INPUT) DIALLEL TABLE (NP X NP)
C
      DIMENSION X(10),Y(10)
      DOUBLE PRECISION VM,CM,AH,X,Y,W
      COMMON W(10,10)
      NA=0
      IF (ME.EQ.0) NA=1
C   NA = TYPE OF ANALYSIS
C        0 = ANALYSIS II
C        1 = ANALYSIS III
      WRITE (IW,90)
      IF (NA.EQ.0) WRITE (IW,160)
      IF (NA.NE.0) WRITE (IW,170)
      VM=0.0
      CM=0.0
      DO 10 I=1,NP
      VM=VM+W(I,I)/FLOAT(NP)
      DO 10 J=1,NP
      IF (I.EQ.J) GO TO 10
      CM=CM+W(I,J)/FLOAT(NP*(NP-1))
   10 CONTINUE
      AH=CM-VM
      IF (ME.GT.2) GO TO 30
      WRITE (IW,100) VM,CM,AH
      DO 20 I=1,NP
      X(I)=W(I,I)-VM
      WRITE (IW,120) I,X(I)
   20 CONTINUE
      GO TO 50
   30 CONTINUE
      VM=0.0
      AH=0.0
      WRITE (IW,110) CM
      DO 40 I=1,NP
   40 X(I)=0.0
   50 CONTINUE
      DO 60 I=1,NP
      Y(I)=0.0
      DO 60 J=1,NP
      IF (I.EQ.J) GO TO 60
      Y(I)=Y(I)+(W(I,J)-CM-(X(I)+X(J))/2.)/FLOAT(NP-1)
   60 CONTINUE
      WRITE (IW,130)
      DO 70 I=1,NP
      Y(I)=Y(I)+Y(I)/FLOAT(NP-2)
      VH=Y(I)
      IF (NA.NE.0) VH=Y(I)+X(I)/2.
      WRITE (IW,120) I,VH
   70 CONTINUE
      WRITE (IW,140)
      DO 80 I=1,NP
      DO 80 J=I,NP
      IF (I.EQ.J) GO TO 80
      W(I,J)=W(I,J)-CM-(X(I)+X(J))/2.-Y(I)-Y(J)
      WRITE (IW,150) I,J,W(I,J)
   80 CONTINUE
      RETURN
   90 FORMAT ('0GARDNER-EBERHART CONSTANTS')
  100 FORMAT ('0VARIETY MEAN',F15.4/' CROSS MEAN',F17.4/' AVERAGE HETERO
     &SIS',F10.4//' VARIETY CONSTANTS'/' PARENT',5X,'CONSTANT')
  110 FORMAT ('0CROSS MEAN',F17.4)
  120 FORMAT (' ',I5,F14.4)
  130 FORMAT ('0VARIETY HETEROSIS CONSTANTS (II) OR GENERAL (III)'/' PAR
     &ENT',5X,'CONSTANT')
  140 FORMAT ('0SPECIFIC HETEROSIS CONSTANTS'/'   CROSS',5X,'CONSTANT')
  150 FORMAT (' ',I2,'  X ',I2,F13.4)
  160 FORMAT ('0ANALYSIS II'/)
  170 FORMAT ('0ANALYSIS III'/)
      END
      SUBROUTINE COV(NP,NR,NS,MO,SMA,SMD,SMR,BMT,EMS,AI,DI,BTI,IE,PP,PU,
     &PPP)
      PN=NP
      RN=NR
      SN=NS
C  MODEL A DESIGN COMPONENTS OF VARIATION
      CVA=0.0
      CVD=0.0
      CVR=0.0
      VCA=0.0
      VCD=0.0
      VCR=0.0
      HF=0.0
      IF (SMA.GT.EMS) CVA=(SMA-EMS)/2./PN/RN/SN
      IF (SMD.GT.EMS) CVD=(SMD-EMS)*PN**2/2./(PN**2-PN+1.)/RN/SN
      IF (SMR.GT.EMS) CVR=(SMR-EMS)/RN/SN/2.
      VE=SQRT(2.*EMS**2/IE)
      IF (SMA.GT.EMS) VCA=SQRT(2.*CVA**2/AI)
      IF (SMD.GT.EMS) VCD=SQRT(2.*CVD**2/DI)
      IF (SMR.GT.EMS) VCR=SQRT(2.*CVR**2/DI)
      AV=PP*CVA
      DV=PU*CVD+CVR
      GV=DV+AV
      PV=GV+EMS
      VAV=PP*PP*VCA
      VDV=PPP*VCD+VCR
      VGV=VDV+VAV
      VPV=VGV+VE
      IHN=AV/PV*100
      IHB=GV/PV*100
      IF (DV.GT.0.0.AND.AV.GT.0.0) HF=SQRT(DV/AV)
      IF (MO.EQ.2) GO TO 10
      WRITE (IW,20)
      WRITE (IW,30) CVA,VCA,CVD,VCD
      WRITE (IW,40) CVR,VCR
      WRITE (IW,50) EMS,VE,AV,VAV,DV,VDV,GV,VGV,PV,VPV,EMS,VE,IHN,IHB,HF
      RETURN
   10 CONTINUE
C  MODEL II DESIGN COMPONENTS OF VARIATION
      CVA=0.0
      VCA=0.0
      CVI=0.0
      VCVI=0.0
      HF=0.0
      IF (SMA.GT.SMD) CVA=(SMA-SMD)/2./PN/RN/SN
      IF (SMA.GT.SMD) VCA=SQRT(2.*CVA**2/AI)
      IF (BMT.GT.EMS.AND.NR.GT.1.AND.NS.GT.1) CVI=(BMT-EMS)/SN
      IF (BMT.GT.EMS.AND.NR.GT.1.AND.NS.GT.1) VCVI=SQRT(2.*CVI**2/BTI)
      AV=PP*CVA
      GV=DV+AV
      PV=GV+EMS+CVI
      VAV=PP*PP*VCA
      VGV=VDV+VAV
      VPV=VGV+VE+VCVI
      IHN=AV/PV*100
      IHB=GV/PV*100
      IF (DV.GT.0.0.AND.AV.GT.0.0) HF=SQRT(DV/AV)
      WRITE (IW,60)
      WRITE (IW,30) CVA,VCA,CVD,VCD
      WRITE (IW,40) CVR,VCR
      WRITE (IW,50) EMS,VE,AV,VAV,DV,VDV,GV,VGV,PV,VPV,EMS,VE,IHN,IHB,HF
      RETURN
   20 FORMAT ('0','COMPONENTS OF GENETIC VARIANCE (MODEL A)'/)
   30 FORMAT ('0',9X,'GENERAL',F20.4,' +-',F15.4//10X,'HETEROSIS',F18.4,
     &' +-',F15.4/)
   40 FORMAT (' ',9X,'RECIPROCAL',F17.4,' +-',F15.4/)
   50 FORMAT (' ',9X,'ERROR',F22.4,' +-',F15.4////10X,'ADDITIVE',F18.4,'
     & +-',F15.4//10X,'DOMINANCE',F17.4,' +-',F15.4//10X,'GENETIC',F19.4
     &,' +-',F15.4//10X,'PHENOTYPIC',F16.4,' +-',F15.4//10X,'ENVIRONMENT
     &AL',F13.4,' +-',F15.4////10X,'HERITABILITY'//15X,'NARROW',I10,'%'/
     &/15X,'BROAD',I11,'%'///10X,'HETEROSIS FACTOR (SQRT(DOMINANCE/ADDIT
     &IVE))',F10.4/)
   60 FORMAT ('0','COMPONENTS OF GENETIC VARIANCE (MODEL II)'/)
      END
      FUNCTION FPROB(M,N,X)
C     COMPUTES PROBABILITY OF F-STATISTIC X WITH M AND N DEGREES OF
C     FREEDOM IN NUMERATOR AND DENOMINATOR RESPECTIVELY.
      INTEGER A,B
      FPROB=1.0
      IF (X.LE.0..OR.M.LE.0.OR.N.LE.0) RETURN
      A=2*(M/2)-M+2
      B=2*(N/2)-N+2
      W=X*M/N
      Z=1./(1.+W)
      IF (A.NE.1) GO TO 20
      IF (B.NE.1) GO TO 10
      P=SQRT(W)
      Y=0.3183099
      D=Y*Z/P
      P=2.*Y*ATAN(P)
      GO TO 40
   10 P=SQRT(W*Z)
      D=0.5*P*Z/W
      GO TO 40
   20 IF (B.NE.1) GO TO 30
      P=SQRT(Z)
      D=0.5*Z*P
      P=1.-P
      GO TO 40
   30 D=Z*Z
      P=W*Z
   40 Y=2.*W/Z
      J=B+2
      IF (J.GT.N) GO TO 60
      DO 50 K=J,N,2
      D=(1.+A/(K-2.))*D*Z
      IF (A.EQ.1) P=P+D*Y/(K-1)
      IF (A.NE.1) P=(P+W)*Z
   50 CONTINUE
   60 Y=W*Z
      Z=2./Z
      B=N-2
      I=A+2
      IF (I.GT.M) GO TO 80
      DO 70 K=I,M,2
      J=K+B
      D=Y*D*J/(K-2)
      P=P-Z*D/J
   70 CONTINUE
   80 FPROB=1.-P
      RETURN
      END
```

```
METHOD 0 DIALLEL ANALYSIS

DESMODIUM DIALLEL ALL BLOCK ANALYSIS

DRY WEIGHT IN A LEONARD JAR (MG/PL)


ANALYSIS OF VARIANCE


SOURCE OF VARIATION          DF      SUM OF SQUARES      MEAN SQUARES            F          P

GENOTYPES                    14          4.9265              0.3519             8.9547     0.0000

  VARIETIES                   4          0.1062              0.0266             0.6759     0.6099

  VARIETIES VS CROSSES        1          3.7614              3.7614            95.7158     0.0000

  CROSSES                     9          1.0589              0.1177             2.9941     0.0028

    GENERAL (GCA)             4          0.6776              0.1694             4.3106     0.0026

    SPECIFIC (SCA)            5          0.3814              0.0763             1.9409     0.0919

GENOTYPE INTERACTION        112          8.5038              0.0759             1.9321     0.0002

  VARIETIES INTERACTION      32          1.9619              0.0613             1.5601     0.0435

  VARIETIES VS CROSSES INT.   8          0.8969              0.1121             2.8530     0.0059

  CROSSES INTERACTION        72          5.6450              0.0784             1.9951     0.0003

    GENERAL INTERACTION      32          2.7854              0.0870             2.2150     0.0009

    SPECIFIC INTERACTION     40          2.8596              0.0715             1.8192     0.0064

ERROR                       129          5.0694              0.0393
```

METHOD 2 DIALLEL ANALYSIS

DESMODIUM DIALLEL ALL BLOCK ANALYSIS

DRY WEIGHT IN A LEONARD JAR (MG/PL)

ANALYSIS OF VARIANCE

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARES	F	P
GENOTYPES	14	4.9265	0.3519	8.9547	0.0000
GENERAL (GCA)	4	0.4973	0.1243	3.1638	0.0162
HETEROSIS (SCA)	10	4.4292	0.4429	11.2711	0.0000
AVERAGE HETEROSIS	1	3.7614	3.7614	95.7158	0.0000
VARIETY HETEROSIS	4	0.2865	0.0716	1.8226	0.1283
SPECIFIC HETEROSIS	5	0.3814	0.0763	1.9409	0.0919
GENOTYPE INTERACTION	112	8.5038	0.0759	1.9321	0.0002
GENERAL INTERACTION	32	2.1933	0.0685	1.7441	0.0159
HETEROSIS INTERACTION	80	6.3106	0.0789	2.0073	0.0002
AVERAGE INTERACTION	8	0.8969	0.1121	2.8530	0.0059
VARIETY INTERACTION	32	2.5540	0.0798	2.0310	0.0029
SPECIFIC INTERACTION	40	2.8596	0.0715	1.8192	0.0064
ERROR	129	5.0694	0.0393		

```
METHOD 0 DIALLEL ANALYSIS

DESMODIUM DIALLEL ALL BLOCK ANALYSIS

DRY WEIGHT IN A LEONARD JAR (MG/PL)


DIALLEL MEANS

    PARENTS      1        2        3        4        5

         1    1.9534

         2    2.1616   1.9861

         3    2.2876   2.0767   2.0014

         4    2.1821   2.1972   2.2983   1.9470

         5    2.2726   2.2637   2.3532   2.2695   2.0415

 POPULATION MEAN -    2.1528    S.E. -     0.0512
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METHOD 0 DIALLEL ANALYSIS

DESMODIUM DIALLEL ALL BLOCK ANALYSIS

DRY WEIGHT IN A LEONARD JAR (MG/PL)


GARDNER-EBERHART CONSTANTS

ANALYSIS III

VARIETY MEAN          1.9859
CROSS MEAN            2.2363
AVERAGE HETEROSIS     0.2504

VARIETY CONSTANTS
PARENT     CONSTANT
   1        -0.0325
   2         0.0002
   3         0.0155
   4        -0.0389
   5         0.0556

VARIETY HETEROSIS CONSTANTS (II) OR GENERAL (III)
PARENT     CONSTANT
   1        -0.0137
   2        -0.0819
   3         0.0236
   4         0.0007
   5         0.0714

SPECIFIC HETEROSIS CONSTANTS
  CROSS       CONSTANT
 1  X  2       0.0210
 1  X  3       0.0415
 1  X  4      -0.0412
 1  X  5      -0.0213
 2  X  3      -0.1012
 2  X  4       0.0422
 2  X  5       0.0380
 3  X  4       0.0377
 3  X  5       0.0220
 4  X  5      -0.0387
```

```
METHOD 2 DIALLEL ANALYSIS

DESMODIUM DIALLEL ALL BLOCK ANALYSIS

DRY WEIGHT IN A LEONARD JAR (MG/PL)


GRIFFING'S COMBINING ABILITY ANALYSIS (MODEL I)

        PARENT     G.C.A. EFFECTS       G.C.A. VARIANCE       S.C.A. VARIANCE

          1           -0.0152               -0.0004                0.0094
          2           -0.0351                0.0006                0.0056
          3            0.0145               -0.0004                0.0117
          4           -0.0108               -0.0005                0.0007
          5            0.0465                0.0016               -0.0015

        S.E. OF THE G.C.A. EFFECTS -                       0.0158
        S.E. OF THE DIFFERENCES BETWEEN G.C.A. EFFECTS -   0.0250


        S.C.A. EFFECTS

     PARENTS       1         2         3         4         5

          1     -0.1691

          2      0.0590   -0.0966

          3      0.1354   -0.0556   -0.1805

          4      0.0552    0.0903    0.1418   -0.1842

          5      0.0885    0.0995    0.1394    0.0811   -0.2042

        S.E. OF THE PARENTAL S.C.A. EFFECTS -                                    0.0322
        S.E. OF THE CROSS S.C.A. EFFECTS -                                       0.0408
        S.E. OF THE PARENTAL DIFFERENCES (S.C.A. EFFECTS) -                      0.0433
        S.E. OF THE COMMON PARENT CROSS DIFFERENCES (S.C.A. EFFECTS) -           0.0612
        S.E. OF THE DIFFERENT PARENT CROSS DIFFERENCES (S.C.A. EFFECTS) -        0.0558
```

Appendix C. Hayman's analysis of population means computer subroutine.

1: HAYMAN subroutine listing. 211

2: HAYMAN sample output. 214

```
      SUBROUTINE HAPM(IW,X,V,PMS,IE,EMS,NAME,CH,NC,NV)
C  HAYMAN'S ANALYSIS OF POPULATION MEANS
C  VERSION 1.0, JUNE 1979
C  IW   - I/O UNIT FOR WRITE CONTROL (I.E. WRITE(IW,...))
C  X    - VECTOR OF MEANS (ORDER = P1,P2,F1,F2,B1,B2)
C  V    - VECTOR OF ST. DEVIATIONS (ORDER = P1,P2,F1,F2,B1,B2)
C  PMS  - POPULATIONS MEAN SQUARE
C  IE   - ERROR DEGREES OF FREEDOM
C  EMS  - ERROR MEAN SQUARE
C  NAME - NAME OF THE EXPERIMENT (ALPHANUMERIC 20A4)
C  CH   - CHARACTER IDENTIFICATION (ALPHANUMERIC 20A4)
C  NC   - MAXIMUM NUMBER OF 'WORDS' IN 'NAME' AND 'CH'
C  NV   - 0 = UNWEIGHTED, 1 = VARIANCE WEIGHTED
C  REQUIRES SUBROGRAM - FPROB
      DIMENSION X(1),V(1),NAME(1),CH(1)
      DIMENSION A(6,6),Q(6),P(6),R(6),Y(6)
      REAL B(6,6)/1.0,1.0,0.0,1.0,0.0,0.0,1.0,-1.0,0.0,1.0,0.0,0.0,1.0,
     &0.0,1.0,0.0,0.0,1.0,1.0,0.0,0.5,0.0,0.0,0.25,1.0,0.5,0.5,0.25,0.25
     &,0.25,1.0,-0.5,0.5,0.25,-0.25,0.25/
      REAL POP(6)/'P1','P2','F1','F2','BC1','BC2'/
      PSS=PMS*5.
      ESS=EMS*IE
      DO 10 I=1,6
10    B(1,I)=X(I)
C  WEIGHTING BY STANDARD ERROR
      IF (NV.EQ.0) GO TO 30
      DO 20 J=1,6
      DO 20 I=1,6
      B(I,J)=B(I,J)/V(J)
20    CONTINUE
30    CONTINUE
C  COMPUTE CORRELATIONS
      DO 50 I=1,6
      DO 50 J=I,6
      TX=0.0
      TY=0.0
      SX=0.0
      SY=0.0
      SXY=0.0
      A(I,J)=0.0
      DO 40 K=1,6
      TX=TX+B(I,K)
      TY=TY+B(J,K)
      SX=SX+B(I,K)**2
      SY=SY+B(J,K)**2
      SXY=SXY+B(I,K)*B(J,K)
40    CONTINUE
      SMX=SX-TX**2/6.
      SMY=SY-TY**2/6.
      SMXY=SXY-TX*TY/6.
      A(I,J)=SMXY/SQRT(SMX*SMY)
      A(J,I)=A(I,J)
50    CONTINUE
C  COMPUTE MULTIPLE-R SQUARED AND SUMS OF SQUARES
      AUP=1.0
      R(1)=1.0
      DO 110 L=2,6
      K=L
      IF (L.LT.4) GO TO 70
C  FINDS AND ENTERS THE INTERACTION COMPONENT WITH THE
C  LARGEST PARTIAL CORRELATION WHEREAS THE MAIN EFFECTS
C  ARE ENTERED FIRST DIRECTLY
      AM=0.0
      DO 60 M=4,6
      VC=(A(1,M)*A(M,1))/A(M,M)
      IF (VC.LE.AM) GO TO 60
      K=M
      AM=VC
60    CONTINUE
70    PE=A(K,K)
      DO 80 I=1,6
      Q(I)=A(I,K)
      P(I)=A(K,I)
80    CONTINUE
      DO 90 I=1,6
      DO 90 J=1,6
      A(I,J)=A(I,J)-(Q(I)*P(J))/PE
90    CONTINUE
      DO 100 I=1,6
      A(K,I)=P(I)/PE
```

```
100   CONTINUE
      R(K)=AUP-A(1,1)
      Y(K)=PSS*R(K)
      AUP=A(1,1)
110   CONTINUE
C  COMPUTE ANOVA COMPONENTS
      EPSS=Y(4)+Y(5)+Y(6)
      ERSQ=R(4)+R(5)+R(6)
      EPMS=EPSS/3.
      PF=PMS/EMS
      PP=FPROB(5,IE,PF)
      AF=Y(2)/EMS
      AP=FPROB(1,IE,AF)
      DF=Y(3)/EMS
      DP=FPROB(1,IE,DF)
      EPF=EPMS/EMS
      EPP=FPROB(3,IE,EPF)
      AAF=Y(4)/EMS
      AAP=FPROB(1,IE,AAF)
      ADF=Y(5)/EMS
      ADP=FPROB(1,IE,ADF)
      DDF=Y(6)/EMS
      DDP=FPROB(1,IE,DDF)
      WRITE (IW,130)
      WRITE (IW,140) (NAME(I),I=1,NC)
      WRITE (IW,140) (CH(I),I=1,NC)
      IF (NV.EQ.1) WRITE (IW,150)
C  PRINT MEANS
      WRITE (IW,160)
      DO 120 I=1,6
      WRITE (IW,170) POP(I),X(I)
      IF (NV.EQ.0) GO TO 120
      WRITE (IW,180) V(I)
120   CONTINUE
C  PRINT ANOVA TABLE
      WRITE (IW,190)
      WRITE (IW,200) PSS,PMS,PF,PP,R(1)
      WRITE (IW,210) Y(2),Y(2),AF,AP,R(2)
      WRITE (IW,220) Y(3),Y(3),DF,DP,R(3)
      WRITE (IW,230) EPSS,EPMS,EPF,EPP,ERSQ
      WRITE (IW,240) Y(4),Y(4),AAF,AAP,R(4)
      WRITE (IW,250) Y(5),Y(5),ADF,ADP,R(5)
      WRITE (IW,260) Y(6),Y(6),DDF,DDP,R(6)
      WRITE (IW,270) IE,ESS,EMS
      RETURN
130   FORMAT ('1HAYMANS ANALYSIS OF POPULATION MEANS'/'  (ADJUSTED FOR M
     &EAN EFFECTS)')
140   FORMAT ('0',20A4)
150   FORMAT ('0WEIGHTED (STANDARD ERROR) ANALYSIS')
160   FORMAT ('-POPULATION MEANS'/)
170   FORMAT (' ',3X,A4,F10.4)
180   FORMAT ('+',20X,'+-',F10.4)
190   FORMAT ('-ANALYSIS OF VARIANCE '//' SOURCE',8X,'DF',14X,'SS',18X,'
     &MS',19X,'F',19X,'P',18X,'RSQ')
200   FORMAT ('0POPULATIONS',4X,'5',5F20.4)
210   FORMAT ('0  ADDITIVE',5X,'1',5F20.4)
220   FORMAT ('0  DOMINANCE',4X,'1',5F20.4)
230   FORMAT ('0  EPISTASIS',4X,'3',5F20.4)
240   FORMAT ('0    ADD-ADD',4X,'1',5F20.4)
250   FORMAT ('0    ADD-DOM',4X,'1',5F20.4)
260   FORMAT ('0    DOM-DOM',4X,'1',5F20.4)
270   FORMAT ('0ERROR',6X,I5,2F20.4)
      END
      FUNCTION FPROB(M,N,X)
C     COMPUTES PROBABILITY OF F-STATISTIC X WITH M AND N DEGREES OF
C     FREEDOM IN NUMERATOR AND DENOMINATOR RESPECTIVELY.
      INTEGER A,B
      FPROB=1.0
      IF (X.LE.0..OR.M.LE.0.OR.N.LE.0) RETURN
      A=2*(M/2)-M+2
      B=2*(N/2)-N+2
      W=X*M/N
      Z=1./(1.+W)
      IF (A.NE.1) GO TO 20
      IF (B.NE.1) GO TO 10
      P=SQRT(W)
      Y=0.3183099
      D=Y*Z/P
      P=2.*Y*ATAN(P)
      GO TO 40
```

```
10      P=SQRT(W*Z)
        D=0.5*P*Z/W
        GO TO 40
20      IF (B.NE.1) GO TO 30
        P=SQRT(Z)
        D=0.5*Z*P
        P=1.-P
        GO TO 40
30      D=Z*Z
        P=W*Z
40      Y=2.*W/Z
        J=B+2
        IF (J.GT.N) GO TO 60
        DO 50 K=J,N,2
        D=(1.+A/(K-2.))*D*Z
        IF (A.EQ.1) P=P+D*Y/(K-1)
        IF (A.NE.1) P=(P+W)*Z
50      CONTINUE
60      Y=W*Z
        Z=2./Z
        B=N-2
        I=A+2
        IF (I.GT.M) GO TO 80
        DO 70 K=I,M,2
        J=K+B
        D=Y*D*J/(K-2)
        P=P-Z*D/J
70      CONTINUE
80      FPROB=1.-P
        RETURN
         END
```

```
BAYMANS ANALYSIS OF POPULATION MEANS
 (ADJUSTED FOR MEAN EFFECTS)

EXPERIMENT 1, 1978

COTYLEDON AREA (MM SQUARED)

VARIANCE WEIGHTED ANALYSIS


POPULATION MEANS

    P1     29.0000
+                      +-     5.0000
    P2     40.0000
+                      +-     7.0000
    F1     41.0000
+                      +-     5.0000
    F2     39.0000
+                      +-     7.0000
    B1     34.0000
+                      +-     6.0000
    B2     41.0000
+                      +-     9.0000

ANALYSIS OF VARIANCE

SOURCE        DF         SS             MS              F             P           RSQ

POPULATIONS    5      8340.0000      1668.0000       35.4893       0.0000       1.0000

  ADDITIVE     1       886.6338       886.6338       18.8645       0.0000       0.1063

  DOMINANCE    1      5008.3594      5008.3594      106.5608       0.0000       0.6005

  EPISTASIS    3      2279.1465       759.7153       16.1642       0.0000       0.2733

    ADD-ADD    1      2152.1638      2152.1638       45.7907       0.0000       0.2581

    ADD-DOM    1        94.0675        94.0675        2.0014       0.1577       0.0113

    DOM-DOM    1        32.9154        32.9154        0.7003       0.4030       0.0039

ERROR        614     28858.0000        47.0000
```

```
HAYMANS ANALYSIS OF POPULATION MEANS
 (ADJUSTED FOR MEAN EFFECTS)

EXPERIMENT 1, 1978

COTYLEDON AREA (MM SQUARED)


POPULATION MEANS

   P1      29.0000
   P2      40.0000
   F1      41.0000
   F2      39.0000
   B1      34.0000
   B2      41.0000

ANALYSIS OF VARIANCE

SOURCE        DF            SS              MS              F             P            RSQ

POPULATIONS    5       8340.0000       1668.0000        35.4893        0.0000        1.0000

  ADDITIVE     1       5977.6563       5977.6563       127.1842        0.0000        0.7167

  DOMINANCE    1       2185.3013       2185.3013        46.4958        0.0000        0.2620

  EPISTASIS    3        176.8449         58.9483         1.2542        0.2893        0.0212

    ADD-ADD    1         48.3907         48.3907         1.0296        0.3107        0.0058

    ADD-DOM    1         63.9702         63.9702         1.3611        0.2438        0.0077

    DOM-DOM    1         64.4840         64.4840         1.3720        0.2420        0.0077

ERROR        614      28858.0000         47.0000
```

Appendix D. Publications.

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STRAIN-SPECIFIC INEFFECTIVE NODULATION OF THE TROPICAL LEGUME *DESMODIUM* BY *RHIZOBIUM*[1]

B. R. Pinchbeck[2]

ABSTRACT

The *Rhizobium* strain CB1789 originally isolated from *Desmodium sandwicense* E. Mey produces a strain-specific ineffective nodulation response with several lines of *D. aparines* (Link) D. C. Study of F_2 and backcross populations from *D. sandwicense* × *D. aparines* hybrids provided evidence for segregation of a dominant gene at a single locus conditioning ineffective nodulation in *D. aparines*. The *D. aparines* lines expressing ineffective nodulation are used in commerce.

Additional index words: **Genetics, Inoculation, N fixation.**

INEFFECTIVE nodulation of soybeans [*Glycine max* (L.) Merr.] by *Rhizobium japonicum* (Kirchner) Buchanan has been shown to be controlled by the three dominant genes Rj_2, Rj_3, and Rj_4 (2, 7, 8). Major genes conditioning strain-specific nodulation have been reported in *Trifolium pratense* L. (6) and *T. subterraneum* L. (3). In preliminary studies with the tropical legume, *Desmodium*, an ineffective nodulation interaction was found for several lines of *D. aparines* (Link) D.C. [*D. intortum* (Mill.) Urb.] when inoculated with *Rhizobium* strain CB1789[3] (Table 1). Strain CB627, referred to in Table 1, is a commercial inoculum for *Desmodium* and effectively induced nodulation of all lines studied. Strain CB1789 was isolated from *D. sandwicense* E. Mey and strain CB627 was isolated from *D. aparines*. Strains CB627 and CB1789, members of the cowpea group of *Rhizobium*, are slow-growing; however, strain CB627 is relatively faster growing than strain CB1789 (J.M. Vincent, personal communication).

Table 1. Qualitative interactions of *Desmodium* lines with two *Rhizobium* strains.

Species†	Effectiveness‡	
	CB627	CB1789
D. sandwicense (CPI18156)	E	E
D. sandwicense (CPI18163)	E	E
D. sandwicense (N993)	E	E
D. sandwicense (CSL)	E	E
D. sandwicense (CWF)	E	E
D. sandwicense (CLL)	E	E
D. aparines (CPI23189)	E	I
D. aparines (Q8382)	E	I
D. aparines (CPI18010)	E	I
D. aparines (Desica-1)	E	E
D. aparines (CPI33814)	E	E
D. pringlei (CPI38722)	E	E
D. uncinatum (CPI8990)	E	E
D. sp. (Ecuador)	E	E§

† Accession or line identification. ‡ E—effective nodulation (green), I—ineffective nodulation (yellow-chlorotic). § Strain induced chlorotic reaction that appears to be pH sensitive.

This study was undertaken to determine the mode of inheritance of the ineffective nodulation interaction between *D. aparines* and Strain CB1789.

MATERIALS AND METHODS

F_2 populations were produced from a *D. sandwicense* male-sterile line derived from CPI18156 (5) which was crossed to the *D. aparines* lines CPI23189 and Q8382. Backcrosses were obtained by crossing the F_1 hybrid onto the male-sterile line. Crosses between *D. sandwicense* and *D. aparines* produce fully fertile progeny and are considered to be closely allied species (4).

The F_2 and backcross plants were grown successively in "modified" Leonard jar assemblies (9) under N-free growth conditions and inoculated with 5 ml of a suspension of strain CB1789. Uninoculated controls and parental genotypes were placed adjacent to the experiment. Eight weeks following planting, the plants were scored for green vs. yellow-chlorotic color induced by N deficiency. Seventy randomly chosen F_2 plants were removed from the Leonard jars, inspected for nodulation response and replanted in pots to check their response to N fertilizer.

RESULTS AND DISCUSSION

The nodulation scores of the F_2 and backcross plants are presented in Table 2. The ineffectively nodulated plants were extremely stunted and chlorotic at 8 weeks and were similar in appearance to the uninoculated and ineffectively nodulated controls. The roots of the ineffectively nodulated plants exhibited various degrees of nodule formation. The majority of nodules were small, whereas a few were of normal size. All nodules sampled lacked pink pigmentation. When these ineffectively nodulated plants were transferred to pots, all responded to N fertilization.

Chi-square analysis of the segregation data support the hypothesis that the strain-specific ineffective nodulation interaction between lines of *D. aparines* and strain CB1789 is produced by a dominant gene at a

Table 2. Observed nodulation scores and Chi-square probability of F_2 and backcross plants inoculated with strain CB1789.

Cross	F_2			Backcross		
	Nodulation		Chi-square probability 3:1	Nodulation		Chi-square probability 1:1
	Ineffective	Effective		Ineffective	Effective	
D. sandwicense (CPI 18156) × *D. aparines* (CPI 23189)	88	36	0.80–0.70	35	36	0.95–0.90
D. sandwicense (CPI 18156) × *D. aparines* (Q 8382)	91	24	0.80–0.70	41	32	0.50–0.40
Deviation χ^2	179	60	0.99–0.95	76	68	0.70–0.60
Heterogeneity χ^2			0.30–0.20			0.60–0.50

---

[1] This investigation was supported by a Ph.D. research grant from the International Development Research Ctr., Ottawa, Canada. Received 3 June 1977.

[2] Statistical Analyst, Computing Services, Univ. of Alberta, Edmonton, Canada (formerly graduate student, Plant Breeding Institute, Univ. of Sydney, Sydney, Australia).

[3] Commonwealth of Australia identification number.

single locus. These results are similar to those reported for soybeans (2, 7, 8).

The symbols N_1/n_1 are suggested for the designation of this ineffective nodulation gene. The *Desmodium* lines which are effectively nodulated have the genotype n_1n_1. The ineffectively nodulated lines would be expected to have the genotype N_1N_1; however, further experimentation would be required to test for functional allelism between the two *D. aparines* lines studied. The ineffectively nodulated *D. aparines* lines have small seeds while effectively nodulated *D. aparines* lines have larger seeds. The linkage of the N_1 allele with small seed size has not been studied.

One ineffectively nodulated line of *D. aparines* has attracted interest as the commercial cultivar – 'Greenleaf' (1). Ineffective nodulation alleles carried by this cultivar may be at a disadvantage if strain CB1789 were to attain a dominant status in the field.

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